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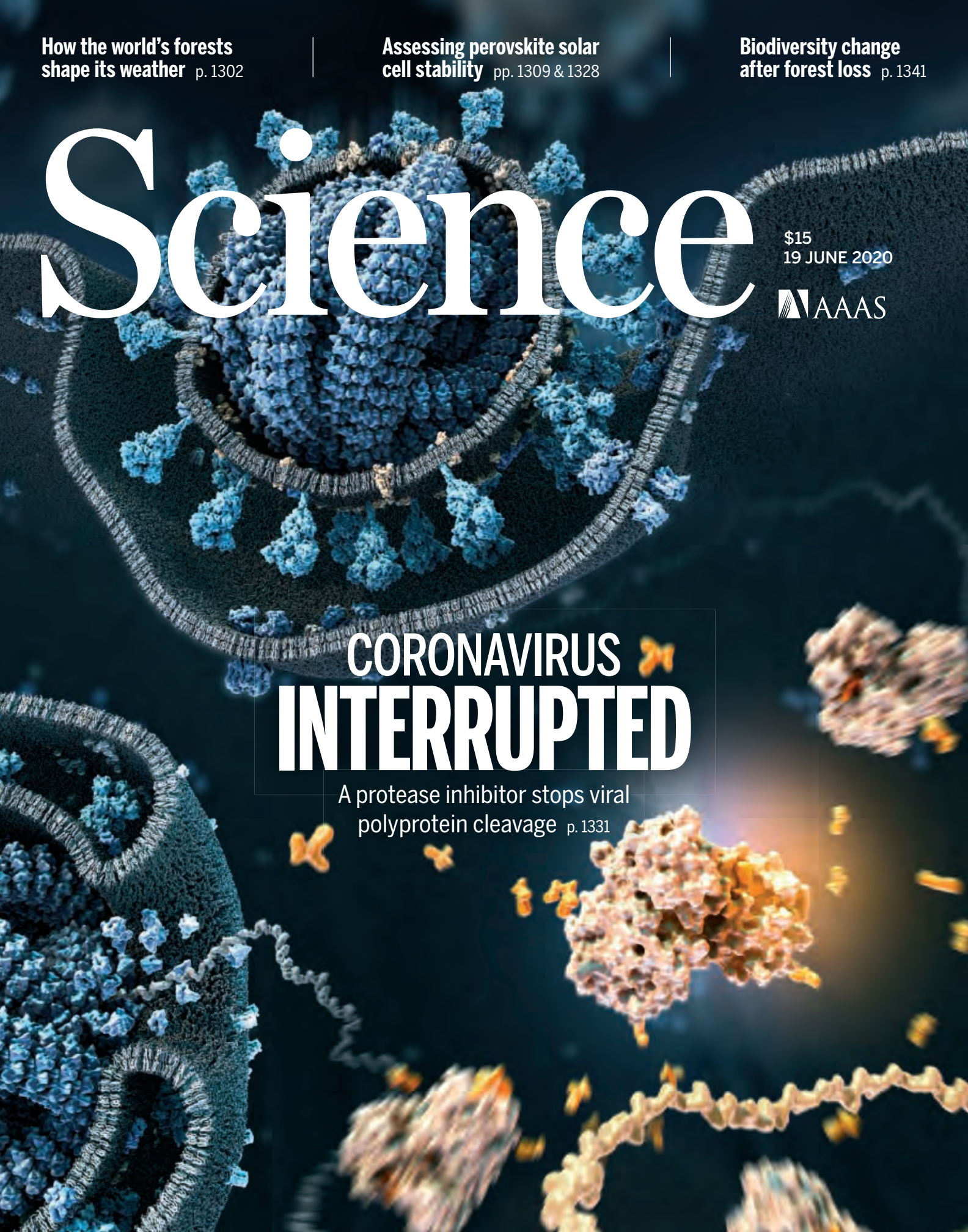

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2019 winners (from left): Longzhi Tan, Zibo Chen, Barbara Klump and Humsa Venkatesh.

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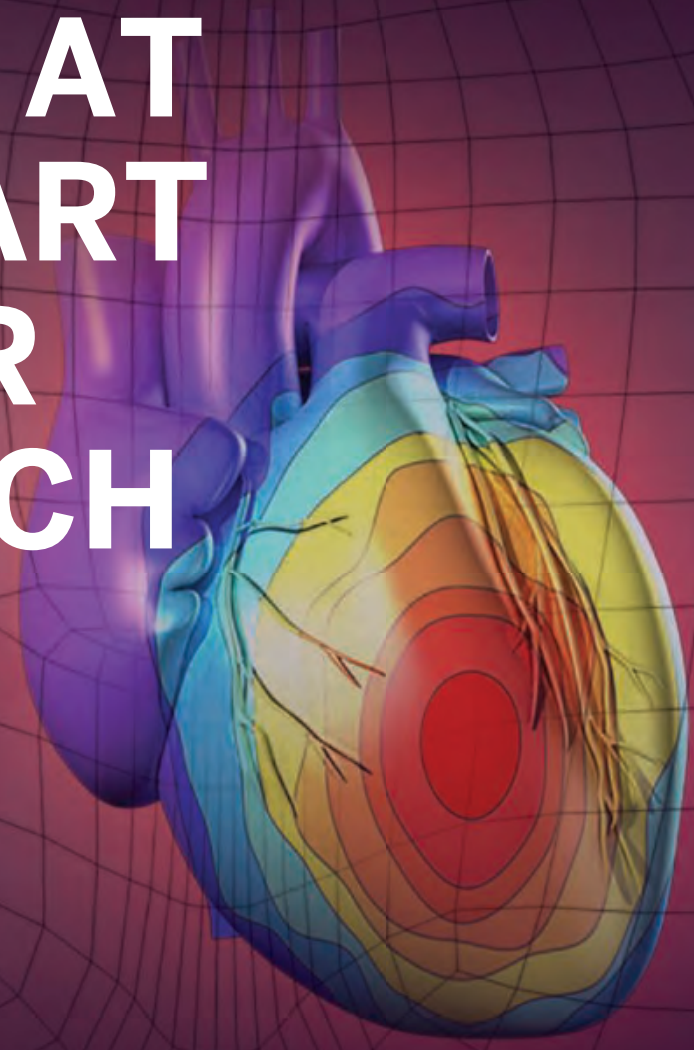

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ON THE COVER

Upon infection, SARS-CoV-2 (blue) uses host machinery to produce polyproteins for replication. The viral main protease (yellow) plays a key role in polyprotein cleavage and thus is a potential drug target. Researchers have designed two antiviral compounds that inhibit activity of the main protease to prevent viral replication. Preclinical studies show that these compounds are promising therapeutic candidates. See page 1331. *Illustration: C. Bickel/*Science; *Data: PDB ID 6M0K (SARS-CoV-2 main protease)*

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EDITORIAL

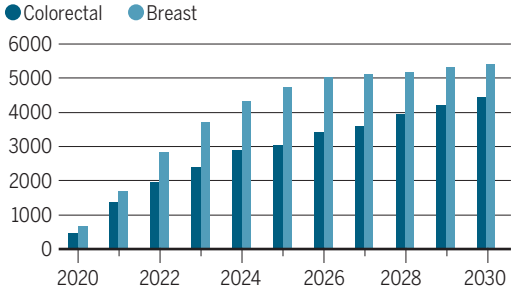
COVID-19 and cancer

Norman E. Sharpless is director of the U.S. National Cancer Institute, Bethesda, MD, USA. norman.sharpless@nih.gov

With the spread of coronavirus disease 2019 (COVID-19), countries and states have instituted lockdowns. These decisions have been difficult and are sometimes described as benefiting the public health at the expense of the economy. Fear of contracting the coronavirus in health care settings has dissuaded people from screening, diagnosis, and treatment for non–COVID-19 diseases. The consequences for cancer outcomes, for example, could be substantial. What can be done to minimize this effect?

Cancer is a complex set of diseases whose prognoses are influenced by the timing of diagnosis and intervention. In general, the earlier one receives cancer treatment, the better the results. There already has been a steep drop in cancer diagnoses in the United States since the start of the pandemic, but there is no reason to believe the actual incidence of cancer has dropped. Cancers being missed now will still come to light eventually, but at a later stage ("upstaging") and with worse prognoses. At many hospitals, so-called "elective" cancer treatments and surgeries have been deprioritized to preserve clinical capacity for COVID-19 patients. For example, some patients are receiving less intense chemotherapy and/or radiotherapy, and in other cases, patients' operations to remove a newly detected tumor are being delayed. There can be no doubt that the COVID-19 pandemic is causing delayed diagnosis and suboptimal care for people with cancer.

Modeled cumulative excess deaths from colorectal and breast cancers, 2020 to 2030*



What will be the likely impact of the pandemic on cancer mortality in the United States? Modeling the effect of COVID-19 on cancer screening and treatment for breast and colorectal cancer (which together account for about one-sixth of all cancer deaths) over the next decade suggests almost 10,000 excess deaths from breast and colorectal cancer deaths; that is, a ~1% increase in deaths from these tumor types during a period when we would expect to see almost 1,000,000 deaths from these two diseases types.* The number of excess deaths per year would peak in the next year or two. This analysis is conservative, as it does not consider other cancer types, it does not account for the additional nonlethal morbidity from upstaging, and it assumes a moderate disruption in care that completely resolves after 6 months. It also does not account for regional variations in the response to the pandemic, and these effects may be less severe in parts of the country with shorter or less severe lockdowns.

Beyond clinical care, the COVID-19 pandemic has caused an unprecedented disruption throughout the cancer research community, shuttering many labs and slowing down cancer clinical trial operations. Many scientists and clinicians are pivoting their cancer research activities to study the impact of SARS-CoV-2 on cancer. The scientific community must ensure that this pause is only temporary, because trials are the only way to make progress in developing new therapies for cancer. Given the long timeline between basic cancer research and changes to cancer care, the effects of pausing research today may lead to slowdowns in cancer progress for many years to come.

Collective action by the clinical and research communities and by governmental agencies can mitigate this potentially substantial impact. The U.S. National Cancer Institute (NCI), for example, has started to address this challenge (see www.cancer.gov). The NCI has worked with the U.S. Food and Drug Administration to increase flexibility and support for clinical trials during the pandemic. For example, allowances have been made to accept "remote" informed consent, and other protocol deviations. In addition, the NCI has announced several new clinical trials and funding opportunities aimed at addressing the relationship between COVID-19 and cancer. Of particular note is the NCI COVID-19 in Cancer Patients Study, a prospective longitudinal study that will collect blood samples, imaging, and other data to understand how COVID-19 affects cancer patients.

Clearly, postponing procedures and deferring care as a result of the pandemic was prudent at one time, but the spread, duration, and future peaks of COVID-19 remain unclear. However, ignoring life-threatening non–COVID-19 conditions such as cancer for too long may turn one public health crisis into many others. Let's avoid that outcome.

–Norman E. Sharpless

*See supplementary materials (science.sciencemag.org/content/368/6497/1290/suppl/DC1).

10.1126/science.abd3377

CREDITS: (PHOTO) NATIONAL CANCER INSTITUTE; (GRAPH) V. ALTOUNIAN/SCIENCE; (DATA) NATIONAL CANCER INSTITUTE

Combating sexual harassment

Sexual harassment, including gender harassment, presents an unacceptable barrier that prevents women from achieving their rightful place in science, and robs society and the scientific enterprise of diverse and critical talent. As the largest single funder of biomedical research in the world, the U.S. National Institutes of Health (NIH) bears a responsibility to take action to put an end to this behavior. In 2019, the NIH began to bolster its policies and practices to address and prevent sexual harassment. This included new communication channels to inform the agency of instances of sexual harassment related to NIH-funded research. This week, the NIH announces a change that will hold grantee institutions and investigators accountable for this misconduct, to further foster a culture whereby sexual harassment and other inappropriate behaviors are not tolerated in the research and training environment.

Last year, an Advisory Committee to the Director (ACD) of the NIH presented a report and recommendations to end sexual harassment. A major theme of this report was the need for increased transparency and accountability in the reporting of professional misconduct, especially sexual harassment. The cases of sexual harassment that surfaced in the wake of the U.S. National Academies of Sciences, Engineering, and Medicine (NASEM) 2018 report highlighted a substantial gap in the NIH's oversight of the research enterprise: There was no straightforward mechanism for the agency to learn of sexual harassment or other misconduct taking place at grantee institutions in the context of NIH-funded research. It was not uncommon for the NIH to discover such cases through the media, amid rightful public outcry. Holding institutions and investigators accountable for this behavior was challenging.

Over the past year, the NIH has established communication channels for anyone—institutions, targets of harassment, bystanders—to inform the agency about instances when sexual harassment compromised the conduct of an NIH-funded project at a grantee institution. Although voluntary provision of information is important and has improved awareness of, and increased NIH actions on, sexual harassment cases, it does not fully cover issues related to the required reporting of a change in principal investigator (PI) or the transfer of a grant by an NIH grantee institution. Two important areas required more attention: differentiating between instances where an institution removed a PI from a grant because of findings or concerns of sexual harassment from other reasons (such as medical leave or job change); and preventing "passing the harasser," in which a scientist who changed institutions could evade the consequences of findings of sexual harassment.

To close these gaps, the NIH has issued new guidance to grantees that sets clear expectations for reporting to the NIH when a grantee institution has a finding of sexual harassment of a PI named on an NIH grant. The notice also makes it clear that the NIH expects its grant recipients who request changes in either investigators or movement of a grant to a new recipient institution to promptly inform the agency, whether changes are related to concerns about safety and/or work environments (e.g., because of concerns about harassment, bullying, retaliation, or hostile working conditions) (see https://grants.nih.gov/grants/guide/notice-files/NOT-OD-20-124.html). This includes situations where a PI, or other senior personnel, are removed from a grant during the investigation of a serious allegation. The NIH will use such information in making decisions related to grant funding, including putting into place appropriate institutional oversight mechanisms or remedies, or deciding whether to grant institutional requests about personnel on grant awards, thus connecting information to direct consequences. This guidance should also unmask NIH-funded harassers who seek to change institutions without revealing their track record, whether the investigator seeks to take an existing grant to a new institution or leaves an existing grant behind for a new PI to take it over. These questions would have to be answered and would reveal any past misconduct to the new institution.

> "...National Institutes of Health (NIH) bears a responsibility to... put an end to this behavior."

The NASEM report noted that risk factors for climates and cultures that fomented sexual harassment included both the perception that reports of sexual harassment would be ignored and the perception that there would be no consequence for investigators. It is our hope that this new guidance sends a clear message to dispel those perceptions.

–Carrie D. Wolinetz, Michael S. Lauer, Francis S. Collins

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NEWS

> “I don’t want more diversity and inclusion seminars. [They] are used to provide a shield to institutions.”
>
> **Astrophysicist Brian Nord** of Fermilab, an organizer of the global #ShutDownSTEM protests on 10 June, calling for effective plans to address racism in science.

Health care workers transport a COVID-19 patient to a port in Manacapuru, Brazil, for transfer to a hospital. The country last week moved into second place for most deaths from the pandemic, behind the United States.

PHOTO: FELIPE DANA/AP PHOTO

IN BRIEF

Edited by **Jeffrey Brainard**

DISPATCHES FROM THE PANDEMIC

FDA backs off malaria drugs

REGULATION | The U.S. Food and Drug Administration (FDA) on 15 June revoked its emergency use authorization for hydroxychloroquine and chloroquine to treat COVID-19. The two antimalaria drugs, touted by President Donald Trump and others as potential game-changers, have failed in recent rigorous clinical trials to prevent disease in newly infected people or help those with symptoms. Former FDA leaders had criticized the agency’s decision to authorize distribution of the drugs from a national stockpile for use on COVID-19 patients, asserting it was based on political pressure, not scientific evidence. The agency said that a review showed the two drugs were “unlikely to be effective in treating COVID-19” and that “serious cardiac adverse events and other serious side effects” outweighed the drugs’ known and potential benefits. FDA’s action still allows the drugs to be used in clinical trials of COVID-19 patients.

Steroid treatment shows promise

BIOMEDICINE | Researchers in the United Kingdom announced this week they have found a cheap, safe drug that could save the lives of patients severely sick with COVID-19: the corticosteroid dexamethasone. The U.K. Recovery trial, one of the biggest efforts to find drugs to treat the disease, included 2104 patients treated with 6 milligrams of dexamethasone for 10 days and compared their outcomes with those of 4321 patients receiving usual care. They found the treatment reduced deaths by one-third in ventilated patients and by one-fifth in patients receiving oxygen only. Scientists announced the results in a press release and said they were working to publish the full results soon.

Antarctic field season postponed

GLACIOLOGY | The U.S. National Science Foundation and the British Antarctic Survey announced last week they would suspend most of their planned Antarctic research during the coming field season to avoid COVID-19 infections. The halt, during Antarctica’s summer from October to March, is especially painful for the International Thwaites Glacier Collaboration, the continent’s largest field project ever. The remote Thwaites is the West Antarctic glacier most at risk of near-term melting. The 2020–21 season was set to be the collaboration’s second on the ice—and perhaps its most critical. So far, both the United States and United Kingdom have prevented infections of the novel coronavirus at their Antarctic stations. Most other nations conducting research on the continent are expected to announce similar steps.

TB, HIV pose some COVID-19 risk

PUBLIC HEALTH | Living with HIV or tuberculosis (TB) increases a person’s likelihood of dying from COVID-19, new data from South Africa suggest—but not

dramatically. An analysis presented in a 9 June webinar by Mary-Ann Davies, a public health expert with the Western Cape government, showed HIV infection raises the risk of dying from COVID-19 by 2.75 times, and TB by 2.58. Those increases are smaller than those posed by other risk factors: The death risk for South Africans in their 50s was 10 times higher than for those younger than 40, for instance, and diabetics were between four and 13 times more likely to die than nondiabetics. South Africa has the world's highest rates of infection with HIV, which undermines the immune system, and TB, which can damage the lungs. Some specialists had worried these vulnerabilities would allow the population to be ravaged by COVID-19.

Colombia coca spraying delayed

DRUG CONTROL | The COVID-19 pandemic has at least temporarily derailed a controversial plan by Colombia's government to resume aerial spraying of a chemical used to kill coca crops that feed the global trade in cocaine. A Colombian court ruled in late May that the spraying of the herbicide glyphosate, which some studies have linked to human health and environmental problems, cannot resume until the government consults with affected communities—a process that has been severely disrupted by the ongoing pandemic. Colombia halted its aerial spraying program in 2015 amid health concerns.

Grant cancellation furor grows

OVERSIGHT | Scientists who counsel National Institutes of Health (NIH) Director Francis Collins have joined a chorus of concerns about the 24 April cancellation of a peer-reviewed grant to study bat coronaviruses in China. Last week, at a meeting of NIH's Advisory Committee to the Director, Baylor College of Medicine geneticist Brendan Lee read a statement from 15 of 17 ACD members. It urged Collins to "thoroughly" review the process that led NIH to kill the grant to the EcoHealth Alliance and consider reversing its decision. NIH acted after conservative politicians and commentators suggested, without evidence, that the coronavirus causing the current pandemic escaped from a lab in Wuhan, China, that received funding from the grant and President Donald Trump said he would end it.

SCIENCEMAG.ORG/TAGS/CORONAVIRUS
Read additional *Science* coverage of the pandemic.

FOREIGN INFLUENCE

NIH probe spurs removal of 54 scientists

The U.S. National Institutes of Health (NIH) says 54 scientists at outside institutions have resigned or been fired as a result of its ongoing investigation into grantees who did not disclose their ties to foreign governments. Michael Lauer, head of extramural research, told a top NIH advisory panel last week that Chinese institutions provided the funding for 93% of the 189 scientists his office has investigated since August 2018. That pool is overwhelmingly Asian (82%) and male (82%); Lauer said China targets Asians in its foreign talent recruitment programs. Some 70% had failed to report receipt of a foreign grant, and 54% their participation in a foreign talent program. Despite fears that these hidden relationships are a vehicle for stealing intellectual property, only 9% of cases involved ties to a foreign company and only 4% had an undisclosed foreign patent.

U.S. nanotechnology lags China

POLICY | The United States should overhaul its research priorities in nanotechnology and bolster funding to restore its leadership in the discipline, a panel of the National Academies of Sciences, Engineering, and Medicine said in a quadrennial review published 9 June. After U.S. dominance in the 2000s, China now produces nearly double the annual number of nanotechnology publications and more than three times the annual number of patents. To regain its edge, the United States needs to upgrade facilities, increase funding, and attract more students to the field, the report says. Coordinating federal nanotechnology efforts, the panel said, is challenging in part because it involves 20 agencies and offices, each with its own budget and priorities.

NOAA chief criticized

LEADERSHIP | The acting administrator of the U.S. National Oceanic and Atmospheric Administration (NOAA), Neil Jacobs, violated scientific integrity policies in September 2019 during the Hurricane Dorian response, an independent report commissioned by the agency has concluded. After President Donald Trump falsely claimed that Alabama was threatened by the hurricane—a weather map was altered accordingly in an incident quickly dubbed "Sharpiegate"—local NOAA forecasters correctly stated it was not. Several days later, under pressure from unnamed officials at the Commerce Department, NOAA's parent agency, Jacobs helped write an unsigned statement that rebuked those staff. That politically driven response, the report concludes, violated the agency's guidelines. Jacobs remains NOAA's acting administrator. NOAA has not fired anyone in response to the violation, instead recommending improved training and policies.

PubMed to add preprints

PUBLICATIONS | To accelerate sharing of research findings, managers of PubMed, the database of biomedical abstracts run by the U.S. National Institutes of Health (NIH), have begun an effort to list draft papers that have not undergone peer review. NIH will add papers posted on preprint servers such as bioRxiv if they cover research supported by its funding. The move follows the agency's 2017 decision to encourage investigators to include preprints in proposals and progress reports. The 1-year pilot will first index the flood of preprints on the COVID-19 pandemic. Users seeking only peer-reviewed articles can exclude preprints from search results.

BY THE NUMBERS

12.4

Average hours U.S. high school biology classes devoted to teaching evolutionary processes in 2019, up 25% from 2007. More than 80% of teachers did not agree with teaching intelligent design as an alternative to Darwinism (*Evolution: Education and Outreach*).

In this fusion reactor's 3-meter-high vacuum chamber, a plasma can reach temperatures above 10 million degrees Celsius.

ENERGY

Fusion lab gets green light for repairs

The Princeton Plasma Physics Laboratory (PPPL) last week received permission from the U.S. Department of Energy (DOE) to begin a $199 million repair of its main fusion energy experiment, a design known as a tokamak. In 2016, a short circuit in a magnetic coil idled the National Spherical Torus Experiment just 10 weeks after a $94 million upgrade. The problem triggered an investigation and a leadership change at PPPL, DOE's sole fusion lab. In 2022, the rebuilt facility is expected to generate plasmas of deuterium, a heavy isotope of hydrogen, allowing researchers to study conditions in which nuclei can fuse and release energy.

Firms halt facial scanner sales

TECHNOLOGY | After widespread global protests about police killings of black people, three technology giants pledged last week to stop selling facial recognition software. The move comes 2 years after researchers and civil rights organizations criticized the technology, citing studies indicating it misclassified darker skinned faces far more often than white ones, putting people of color at increased risk of racially biased policing. After initially disputing the findings, Amazon on 10 June reversed itself and suspended its software sales for 1 year. IBM announced on 9 June it would abandon the sales, and Microsoft on 11 June suspended selling its software until there is a national law regulating the technology.

Strict laws reduce gun deaths

POLICY RESEARCH | States that restrict children's access to guns, don't allow people to carry concealed weapons without a permit, and don't allow lethal "stand your ground" self-defense can expect to have 11% fewer gun deaths every year than states with more permissive gun laws, according to a study this week in the *Proceedings of the National Academy of Sciences*. The three types of gun laws were common enough from 1980 to 2016 to reveal meaningful statistical relationships.

IN OTHER NEWS

MOON ROVER NASA awarded a $200 million contract to Astrobotic Technology for a lander to carry the first rover it will send to the Moon's surface. The robot, which will scout for ice that could supply water for astronauts, is to arrive at the Moon in 2023.

OPEN-ACCESS DEAL The University of California (UC) system has signed the biggest open-access deal in North America with one of the largest commercial scientific publishers, Springer Nature. The publisher agreed to explore making all articles that UC corresponding authors publish in the *Nature* family of journals free to read on publication starting in 2022.

NOMINEE OPPOSED More than 90 scientists asked a U.S. Senate panel not to confirm President Donald Trump's nominee, toxicologist Nancy Beck, as chair of the Consumer Product Safety Commission. The researchers, including former employees of the Environmental Protection Agency, cite decisions she made as head of EPA's chemical safety office about toxic substances that they say put workers at risk.

PHOTO: ELLE STARKMAN/PPPL COMMUNICATIONS/CC BY

IN DEPTH

Mobile teams will help test COVID-19 vaccine candidates, a strategy used to evaluate an experimental Ebola vaccine in the Democratic Republic of the Congo.

PHOTO: DIANA ZEYNEB ALHINDAWI/*THE NEW YORK TIMES*/REDUX

COVID-19

Pandemic vaccines are about to face the real test

U.S. and global efforts are taking different approaches to key efficacy trials of candidates

By **Jon Cohen**

A Chinese company will turn to Brazil for help. The World Health Organization (WHO) is adopting a strategy forged in a war zone during an Ebola outbreak. And the Trump administration plans to lean on existing infrastructure for testing HIV and flu vaccines. These are the disparate strategies about to be employed in the next and most important stage of the COVID-19 vaccine race: the large-scale, placebo-controlled, human trials needed to prove which of the more than 135 candidates are safe and effective.

Two such efficacy trials plan to start next month, even as the United States and global initiatives struggle to answer major questions, from what it means for a COVID-19 vaccine to work to how to find enough people exposed to the virus so a candidate can be put to a real-world test. Populations that have high levels of viral transmission are a moving target—Wuhan, Seattle, or Milan might once have been a good place to test the mettle of a vaccine, but no longer. And quickly enrolling tens of thousands of properly informed people who meet a trial's entry criteria is a "big lift," says Susan Buchbinder, an epidemiologist at the San Francisco Department of Public Health who runs vaccine trials.

Competition among trial efforts could hinder the global push, says Wayne Koff, who heads the nonprofit Human Vaccines Project and formerly led the HIV vaccine program at the National Institute of Allergy and Infectious Diseases (NIAID). "It's absolutely extraordinary how much has been done in 6 months, but there's an old adage that everybody loves to collaborate unless they want to win." Others, however, don't anticipate conflicts, noting that scientists and officials are sharing information about trial designs and plans. "Each one will contribute differently," says Ana Maria Henao Restrepo, lead representative of WHO's vaccine effort, Solidarity. "I don't see competition."

"Winning," one of U.S. President Donald Trump's favorite terms, is the clear goal of Operation Warp Speed, the U.S. project that aims to start to vaccinate millions of Americans in October and offer shots to 300 million people in the United States by January 2021. After winnowing down vaccine candidates in an opaque process over the past month and committing what could be more than $2 billion to its top choices, Warp Speed plans to enter three to five of them into efficacy trials that will have "harmonized" protocols to streamline oversight and will run analyses in central labs so data can more easily be compared.

The first Warp Speed candidate to launch, from the biotech Moderna, is composed of RNA encoding the coronavirus spike protein from SARS-CoV-2, the virus that causes COVID-19. The candidate's efficacy trial, announced by the company on 11 June, will enroll 30,000 people and take place at established HIV and flu vaccine test sites, primarily at U.S. hospitals and universities, now overseen by Warp Speed. But which of those brick-and-mortar sites will have enough SARS-CoV-2 circulating near them to quickly produce an efficacy signal is uncertain given the shifting distribution of new cases in the United States.

China has an even starker problem: There's currently little transmission to speak of in the country, which has forced Sinovac Biotech, a company based in Beijing, to stage efficacy trials of its vaccine candidate in Brazil, where the COVID-19 epidemic is now raging. With a product com-

Science's COVID-19 coverage is supported by the Pulitzer Center.

posed of the entire virus, inactivated with chemicals, Sinovac announced this week it is collaborating with the Butantan Institute, a major research institution in São Paulo that manufactures vaccines. "We are working very hard to start the trial in July," says Sinovac Senior Director Meng Weining.

WHO proposes a different solution for Solidarity's efficacy trials. The agency hasn't yet announced which candidates Solidarity will test, but, unlike Warp Speed—which won't consider Chinese-made vaccines—it is open to products from every country and has made public detailed criteria for how it will prioritize vaccines. To cope with the patchiness of the pandemic, Solidarity will adopt a strategy Henao Restrepo helped develop for Ebola vaccine trials in Guinea in 2015 and, 3 years later, in the Democratic Republic of the Congo (DRC): setting up vaccination teams that can quickly mobilize to localized outbreaks.

"We did this in Congo despite the war," Henao Restrepo says. "It's not the traditional way, and some people think that we are crazy, but we have done it not once but twice." In the DRC, about 20 teams with 15 members each drove around the affected regions and set up temporary sites, vaccinating and following more than 300,000 people.

Warp Speed, which could if needed expand its trials to international sites used for HIV drug and vaccine testing, also plans to form "surge clinics" to quickly recruit people in rural U.S. areas with big outbreaks or pockets of high transmission such as nursing homes. Models driven by machine learning will help Warp Speed forecast where infection will be highest, says Peter Gilbert, a University of Washington, Seattle, biostatistician. "There are risk predictors that account for space and geography and features that are more constant like race, ethnicity, or preexisting conditions," Gilbert says. "It's really complicated."

One of the trickiest issues for trial designers is deciding what, exactly, represents success for a COVID-19 vaccine. "Is it an infection endpoint, a transmission endpoint, preventing moderate disease, or preventing severe disease?" Koff asks.

"There was a lot of debate on that question," for Warp Speed, notes John Mascola, who heads NIAID's Vaccine Research Center and contributes to the project. A COVID-19 vaccine that fails to prevent infection might still provide great benefit if it reduces symptomatic disease, so Warp Speed and Solidarity both ultimately chose that as the primary endpoint of the trials. Trial volunteers who develop fever, headache, dry cough, or other symptoms linked to COVID-19 will be tested for SARS-CoV-2, to see whether more people with confirmed infections develop symptomatic disease in the placebo arm of the trial than among those who received the vaccine.

To detect an efficacy signal, both Warp Speed and Solidarity estimate they will need to give each vaccine to 15,000 to 20,000 people in a population that has a 1% per year incidence of SARS-CoV-2 infection. If the vaccine prevents COVID-19 symptoms at least 50% of the time, its efficacy should be clear in 6 months, after about 150 infections have accumulated in the trial.

Both efforts will pit multiple vaccine candidates head to head. One difference is that Solidarity plans to compare all its vaccines against a shared placebo group, an approach that reduces the number of volunteers the researchers need to recruit and follow. "In the Solidarity trial, the philosophy is we have to make this thing really simple," says Gilbert, who has worked with this effort, too. Solidarity trial sites have the option to do nuanced studies on immunity and other issues, but those are built into Warp Speed's trials. They will do repeated blood draws and nasal or throat swabs to evaluate immune responses and viral levels. The data might help researchers understand why vaccines succeed or how they might affect transmission.

> **"We need to be really careful how we manage expectations."**
> **Wayne Koff**, Human Vaccines Project

In addition to Solidarity, WHO is helping the Access to COVID-19 Tools (ACT) Accelerator, another global effort, which may stage its own vaccine efficacy trials if companies do not want to participate in Solidarity. "Companies may or may not be very enthusiastic about head-to-head comparisons," explains Soumya Swaminathan, WHO's chief scientist and top liaison to the ACT Accelerator. And the ACT Accelerator has pockets as deep as Warp Speed: Countries and philanthropies in May pledged $8 billion, with a commitment that it would equitably distribute any proven COVID-19 products—vaccines, treatments, diagnostics—to rich and poor alike.

Buchbinder is impressed by the speed at which these massive efforts have gotten underway. "It's unlike any other research I've undertaken," she says. But she and others are careful to temper expectations. Even though she will oversee a Warp Speed trial site, for example, she doubts the U.S. effort will meet Trump's goal of having a proven vaccine by October. Koff agrees; the failures of so many HIV vaccine trials have sobered him, he says. "We need to be really careful how we manage expectations," he concludes. ■

COVID-19

Can phone apps slow the spread of the coronavirus?

Digital contact tracing is growing—and facing its first real-world tests

By **Kelly Servick**

Health departments around the world are betting on technology to help stem the stealthy spread of the coronavirus: cellphone apps that aim to identify and alert those who recently came into contact with an infected person. By encouraging those potentially exposed to COVID-19 to self-isolate, the thinking goes, a phone app could swiftly cut off chains of transmission. Dozens of local and national governments have launched official apps or are developing them.

"It's very appealing—that you have an app that does all this work," says Hannah Clapham, an epidemiologist at the National University of Singapore. But she and others warn that an app can't replace human contact tracers. "I worry that we think it's going to save us."

So far, only epidemiological models suggest apps can change a pandemic's course. Ensuring that an app detects risky contacts without overwhelming users with false alarms is one challenge; getting enough people to download an app is another. As health officials weigh competing apps and prepare pitches to privacy-conscious citizens, epidemiologists, engineers, and behavioral scientists are considering how to put an app to the test.

People can apparently transmit the coronavirus for days before they develop symptoms, so by the time health departments learn of a case, they have precious little time before infected contacts start to spread the virus. "You have a couple days to chase people down," says C. Jason Wang, a health policy researcher at Stanford University who works with health departments on COVID-19. And traditional contact tracing—interviewing the infected person, tracking down the recent contacts they can recall, and telling those people to self-isolate—is time consuming.

In contrast, phones could detect when two

Millions of people have downloaded apps designed to alert them to coronavirus exposure.

PHOTO: MINZAYAR OO/PANOS PICTURES/PANOS PICTURES/REDUX

users are close enough to share the virus, and an app could alert one when the other gets sick—even if those people are strangers who happened to sit in adjacent subway seats. "The technology response is absolutely necessary," Wang says, "and it needs to be fast."

Some Asian countries that faced early outbreaks have already put smartphone tracking to use. South Korea collects GPS data to publicize recent paths of newly infected people, and last week announced it would require facilities including bars and night clubs to collect identifying information from visitors' phones to share with health authorities. The Chinese government has combined location data with other surveillance to restrict citizens' movement if they may be infected. GPS location tracking is also a component of apps in India, Iceland, and U.S. states including North Dakota and Utah. But GPS technology isn't precise enough to gauge short distances between two phones. And widespread location tracking raises privacy concerns.

Many governments are instead developing apps that rely on short-range Bluetooth radio signals, which can detect close encounters but don't track movements. Each phone broadcasts an ID number to nearby phones that record such "handshakes." If a user has symptoms or tests positive, the app can trigger alerts to those recent contacts.

To be effective, these apps will first have to reliably estimate distance, based on the strength of the received signals. In a test of Singapore's TraceTogether app, computer scientists Douglas Leith and Stephen Farrell at Trinity College Dublin found flaws in those estimates. The study, posted online but not peer reviewed, showed that when people sat across a table, the received signals were much weaker if their phones were in their pockets versus on the table. Sometimes the signal increased as people moved farther apart—perhaps because of reflection off metal surfaces such as supermarket shelves. The imprecision could lead to both missed encounters and false alarms, Leith warns.

Imperfect Bluetooth readouts can still help if interpreted conservatively, says Marcel Salathé, an epidemiologist at the Swiss Federal Institute of Technology Lausanne. He advises the Swiss government on an app now in a pilot phase that aims to detect when someone comes within 2 meters of an infected person for at least 15 minutes. He thinks his team can tune the system so that "if somebody gets an exposure notification, we will feel damn sure it's actually been a contact."

An app can only catch interactions between people who have installed it. Singapore, which launched TraceTogether on 20 March, says roughly one-third of the population has signed on. But uptake of an app "needs to be almost improbably high to really capture all of the contacts that might be relevant," says Allison Black, a genetic epidemiologist at the University of Washington, Seattle, and the Fred Hutchinson Cancer Research Center.

Recent modeling by infectious disease epidemiologist Christophe Fraser and colleagues at the University of Oxford predicted that if about 56% of a population used an app, it alone could reduce the virus' reproduction number—how many people catch the virus from each infected person—enough to control the outbreak. But Salathé says that, even at relatively low levels of uptake, an app could still prevent infection and save lives: "As soon as you have double digits, I think the effect is already quite substantial."

An app's impact depends on whether people stay home when it tells them to. Human contact tracers connect a potentially infected person to social supports that help them self-quarantine—for example by arranging grocery delivery or even a hotel stay. But because most apps keep users anonymous, health officials won't automatically know who gets an alert or what they need to stay home, Wang says. And users might not dutifully check in with health officials when they get an alert. "That's too optimistic," he says. "We tell people to stay at home; they go to the beach."

To test whether an app accurately flags risky encounters, health officials want to know the proportion of identified contacts who end up sick—the "secondary attack rate." As a rule of thumb, if an app's attack rate matches or exceeds that of traditional contact tracing, "we know the app is doing a really good job," Salathé says.

Some app designs allow health officials access to anonymized ID codes of infected users and all their contacts. That means officials can calculate the secondary attack rate and fine-tune the app by checking how many notified users later report symptoms or a positive test through the app. In Norway, which launched such a centralized app in April, municipalities will compare how many, and how quickly, contacts are identified by the app versus through traditional contact tracing, says Emily MacDonald, an epidemiologist at the Norwegian Institute of Public Health.

Other apps, including Switzerland's and one released this week in Germany, are decentralized, meaning data about interactions stay on a phone. Privacy advocates favor this design, and Google and Apple encourage it. Last month, they released technology to support Bluetooth tracking, but only for apps with a decentralized design. With these apps, health departments will know about potentially infected users only if they report getting an alert. Critics say that design could make it harder to evaluate the app's performance.

The ultimate test, some researchers say, is a randomized trial to gauge whether using an app brings down rates of infection. But such trials "would be very costly and difficult," says Oxford behavioral economist Johannes Abeler. Because COVID-19 is relatively rare, such trials would have to be large. And to gauge effectiveness, researchers would have to factor in the proportion of a participant's contacts who had downloaded the app—which might be nearly impossible to know.

Another possibility is to compare changes in infection rates between geographic areas or demographic groups with different levels of app use, suggests Rosalind Eggo, an epidemiologist at the London School of Hygiene & Tropical Medicine who hopes to study the impact of apps as data accumulate. "We have a lot of technology that can help us here," she says. "There's quite a lot of people saying, 'Oh, it won't work.' I think we need to try." ■

COVID-19

U.S. academic research funding stays healthy despite pandemic

Labs reopening even as campuses struggle

By **Jeffrey Mervis**

The coronavirus pandemic has put U.S. higher education in a deep financial hole, triggering hiring freezes, benefit cuts, and a slew of other cost-cutting measures designed to offset huge projected revenue losses. Yet amid the uncertainty about state aid, the supply of foreign students, and other intangibles, one revenue stream has remained healthy: the money universities receive from others—especially the federal government—to carry out research. That fact looms large in any effort to forecast the long-term impact of COVID-19 on U.S. academic research.

In 2018, the federal government provided 53% of the $79 billion spent on research on U.S. campuses, and federal spending has actually increased during the pandemic. This spring, Congress included an additional $3.6 billion for research related to COVID-19 in a string of economic relief packages. Academic leaders are hoping legislators will add to that total in the next stimulus package.

Such developments have put research administrators like Chris Cramer in an enviable position during ongoing budget meetings with other senior academic officers. "These days, I'm the only one who gets to say that, even with large error bars, none of my projected numbers are in the red," says Cramer, who oversees the University of Minnesota's $870 million portfolio of sponsored research.

In part, that's because the pandemic has had little impact on the U.S. government's machinery for awarding grants, even as the number of U.S. COVID-19 cases has surpassed 2 million. At the National Institutes of Health (NIH), which provides about two-thirds of all federal funding for academic research, "We went to 100% virtual peer review [of grant proposals] practically overnight, and it's gone remarkably well," says Michael Lauer, the agency's director of extramural research.

A temporary change in the rules for managing federal grants has also helped buoy academic research. For the past 3 months, institutions have been allowed to continue to pay the salaries of researchers on grants even if their labs have been shuttered.

The 19 March directive, part of a broader strategy to save jobs, reflects the fact that federal grants have always covered much more than the cost of just running experiments or collecting field data. Grantees are also paid for analyzing data, writing papers, supervising students and postdocs, and submitting proposals for new and follow-up studies—work that can all be done from home. (The guidelines were set to expire as *Science* went to press, but could be extended.)

At Johns Hopkins University, which carries out more sponsored research—some $2.5 billion—than any other university in the country, President Ronald Daniels warned this spring of a possible $475 million budget shortfall next year. The bulk of it comes from the suspension of elective procedures at Johns Hopkins's vast network of hospitals and clinics and will be borne by physicians.

The impact on research will be comparatively small, his analysis suggests. A 1-year hiring freeze will save $40 million, Daniels predicted. But the unceasing competition among top research institutions to attract talent and funding all but guarantees that there will be exceptions. "The university must emerge from this crisis stronger, and making strategically important faculty hires is crucial to that end," a university spokesperson says.

Although many institutions are still figuring out what the fall semester will look like for undergraduates, almost all have already begun to reopen their labs. This week the University of Michigan, which ranks second to Johns Hopkins in sponsored research spending, entered the third phase of a four-step plan to reopen, by the end of the month, all 50 of its research buildings, housing some 5000 labs. "There's a bimodal distribution" of faculty attitudes toward the reopening, says Rebecca Cunningham, the school's vice president for research. "Some are trampling down the doors, and others have serious concerns."

At most institutions, some types of research are still off-limits, including work involving human subjects and most field studies. Last week in *Science*, Cunningham and five other senior administrators from major research universities offered guidance to institutions on how to reopen labs safely. Their suggestions include performing health checks at entrances, controlling access, reducing density, revising work schedules, and requiring personal protective equipment and deep cleaning. "It's a new way of working," Cunningham says, "like with grocery shopping. But people understand that it's necessary."

Research will not emerge from the shutdown unscathed. NIH estimates $10 billion has simply "disappeared" from its budget as a result of lost productivity, Director Francis Collins told a Senate panel last month. Universities have also had to shoulder the unanticipated costs of shutting down and restarting research on their campuses.

A 330-member coalition of research advocates wants Congress to add $31 billion to NIH's current $41 billion budget to make up for such losses and to address infrastructure needs at NIH and around the country. Many of the same organizations are also seeking $46 billion in direct payments to universities to recoup lost revenues.

University officials hope the prominent role their scientists are playing in fighting the pandemic will help persuade lawmakers to provide the additional funding. "When have research universities ever been more in the news?" Cramer asks. "We're on the front pages every day." ■

University of Michigan researchers have their temperatures taken as part of new health and safety procedures.

PHOTO: ROGER HART/MICHIGAN PHOTOGRAPHY

ARCHAEOLOGY

Incest in ancient Ireland suggests an elite ruled early farmers

DNA from massive Newgrange tomb reveals a practice linked to royalty around the world

By **Andrew Curry**

Twenty-five kilometers north of Dublin, a masterpiece of Stone Age engineering rises from the hills: a circular structure 12 meters high, almost the area of a U.S. football field, and made up of more than 200,000 tons of earth and stone. Some of the first farmers to arrive in Ireland erected this monument, called Newgrange, nearly 1000 years before Stonehenge or Egypt's first pyramids were built. Archaeologists have assumed it was a ceremonial site and communal tomb—an expression of an egalitarian society.

Now, DNA from a middle-aged man buried in 3200 B.C.E. at the center of this mighty mound suggests otherwise. His genes indicate he had parents so closely related they must have been brother and sister or parent and child.

Across cultures, incest is almost always taboo—except in inbred royal families. Its genetic traces at Newgrange suggest social hierarchy took hold in Ireland earlier than thought, according to a new study. "Maybe we've been arguing too far that [these people were] egalitarian," says Jessica Smyth, an archaeologist at University College Dublin who was not part of the team.

The newly sequenced genomes from Newgrange and other Irish tombs are part of a wider re-evaluation of the Neolithic era, which is marked by the advent of agriculture. Over the past decade, researchers have used ancient DNA to track a slow-motion, 5000-year expansion of ancient farmers from Anatolia across Europe. The Neolithic settlers who arrived in Ireland around 3700 B.C.E. were the westernmost limit of that expansion.

Most Irish Neolithic settlements are small scale, with houses of roughly equal size. As seen in Neolithic graves across Europe, their burials show little sign of hierarchy. Even in major monuments like Newgrange, human remains were jumbled together, as if in a communal tomb. "Archaeologists [have] argued for a long time for a more egalitarian Neolithic," says co-author Thomas Kador, an archaeologist at University College London.

Newgrange is pierced by a passage that leads to a central chamber; its entrance is oriented so a ray of sunlight illuminates the chamber at dawn on the shortest day of the year. "It's clearly a place of public ritual and must have taken a lot of manpower to construct," says geneticist Lara Cassidy of Trinity College Dublin. Hundreds of such passage tomb monuments are found across Ireland.

Most of the bodies in those tombs were cremated. But at the heart of Newgrange, excavators in the 1970s found the unburnt bones of one man, labeled NG10, in a niche decorated with elaborately carved stones. Cassidy and her team were able to extract DNA from that skeleton.

Comparing NG10's DNA and that of other Neolithic burials with DNA from people living on the island centuries earlier shows Neolithic farmers arrived in Ireland as part of a mass migration, and soon swamped or eliminated the genetic legacy of earlier hunter-gatherers, says geneticist Daniel Bradley, a co-author also at Trinity College Dublin.

NG10's DNA also reveals his unusual parentage. In a paper this week in *Nature*, Cassidy's team draws on parallels in the historical record to argue that the son of an incestuous union buried in such a prominent tomb points to a hereditary ruling class. "Matings like that are taboo pretty much universally, with very few exceptions," he says.

Those exceptions include Egyptian pharaohs, who were considered deities who needed to marry each other. Royal siblings in Hawaii and the Incan empire were also known to marry, concentrating power in one family. "I believe we're seeing a similar social dynamic at play among colonists of Neolithic Ireland," Cassidy says.

Early Irish royalty may have been buried in Newgrange tomb, shown at dawn on the winter solstice.

Additional DNA from more than 40 people buried at other Neolithic sites, including three passage tombs, supports the existence of a close-knit elite. People buried in passage tomb sites were more closely related to each other than to people buried in other types of tombs, even though the passage tombs were separated by hundreds of kilometers and spanned more than 500 years. Some individuals in the far-flung passage tombs could have been second or third cousins or great-great-great-great-grandparent and child.

Chemical isotopes in their bones show the people in the passage tomb burials ate more meat and animal products than their contemporaries. The burials also include women and children, suggesting social status was inherited rather than won in a single lifetime. "We're talking about something that could be inherited," Kador says. "A small [related] elite called the shots, like in Egypt."

But other archaeologists are cautious. "To go from [NG10] to saying these are proto-state societies where you have a godlike elite is pushing it a bit far," says University of Manchester archaeologist Julian Thomas. "It's one guy." He notes Newgrange was a burial place for almost 1000 years, too long to make generalizations from a single burial.

One way to settle the debate is to look at similar passage tombs built on the Orkney Islands and in Wales and France. "The question is whether this arose in Ireland or whether they were importing existing social structures into the island," Cassidy says. "It's going to be very exciting to see if this is a pattern we see in other areas." ■

Andrew Curry is a journalist in Berlin.

PHOTO: KEN WILLIAMS/SHADOWSANDSTONE.COM

In late 2019, reporters visited the Arak Heavy Water Complex, a potential source of plutonium.

NUCLEAR PROLIFERATION

New tensions dim hopes for salvaging Iran nuclear deal

Monitoring agency seeks clarity about clandestine R&D

By **Richard Stone**

Since U.S. President Donald Trump pulled the United States out of the Iran nuclear deal 2 years ago, other signatories have tried to salvage the agreement in hopes of constraining Iran's ability to resurrect a nuclear weapons program. They haven't given up yet. And although Iran has resumed activities proscribed by the deal, including stepping up uranium enrichment, it has kept the door open to a reversal. But a series of disputes has continued to fray the agreement.

This week, questions about Iran's past clandestine atomic research program—including the disappearance of a missing uranium disk that could be used in a bomb, and illicit explosives testing—took center stage during a meeting of the governing board of the International Atomic Energy Agency (IAEA). And recent moves by the Trump administration have threatened to derail the conversion of Iranian nuclear facilities to reduce the risk they could contribute to a weapons program.

The Joint Comprehensive Plan of Action (JCPOA), as the 2015 deal is called, offered Iran relief from economic sanctions in exchange for dismantling large pieces of its nuclear program. Although experts generally agree the JCPOA worked, the Trump administration withdrew in May 2018, arguing it didn't go far enough. New U.S. sanctions have since battered Iran's economy.

"It hasn't been easy to watch the deal get dismantled," says a European diplomat who requested anonymity because talks with Iran are at a sensitive stage. Yet, "So far, Iran has been notably measured in building up its nuclear capabilities," Christopher Ford, assistant secretary for international security and nonproliferation at the U.S. State Department, recently told journalists.

An IAEA report shared with the governing board last week said Iran has been enriching uranium hexafluoride gas to 4.5% of the fissile isotope uranium-235 (U-235) over the past year. By 20 May, it had stockpiled 1572 kilograms of enriched uranium, ostensibly for use in civilian reactors. Nuclear bombs require enrichment levels exceeding 90% of U-235. "They could have made a lot more uranium at a higher enrichment level, but they haven't," says Richard Johnson of the nonprofit Nuclear Threat Initiative.

But Iran has greatly ramped up R&D on advanced centrifuges, which could speed up enrichment and reduce the "breakout time" needed to enrich a bomb's worth of weapons-grade uranium to 3 months. The JCPOA ensured the breakout time would exceed 1 year; the Western diplomat estimates it is still more than 6 months.

IAEA and outside analysts have also been poring over a cache of documents Israeli agents spirited out of Iran in early 2018. The nuclear archive, as it's called, has yielded fresh insights into Iran's past R&D on nuclear weapons and its plans for underground testing that IAEA says call into question the "correctness and completeness" of declarations Iran made in 2003, when it agreed to come clean on its nuclear program and permit inspectors broad access to sites.

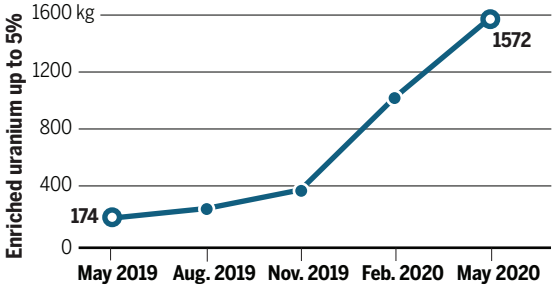
Iran had hoped the deal would end questions about its shuttered bomb effort. But IAEA now wants to know the whereabouts of a disk of uranium metal that could be used to generate neutrons for triggering fission in a bomb's U-235 core. Evidence suggests it was housed at a site called Lavizan-Shian in Tehran, which Iran razed and sanitized in 2003 and 2004. In January, IAEA asked Iran to give its inspectors access to two unnamed sites to verify the absence of undeclared nuclear material and activities. According to a report last week from the nonprofit Institute for Science and International Security, one site appears to be a testing range for high explosives near Abadeh, in central Iran. During a September 2019 press conference, Israeli Prime Minister Benjamin Netanyahu showed satellite images indicating that site was razed only in July 2019.

In a 28 January letter to IAEA, Iran rebuffed the agency, declaring it "will not recognize any allegation on past activities and does not consider itself obliged to respond to such allegations." The dispute has raised fresh questions about Iran's intentions and gives the United States another cudgel to try to persuade the United Nations Security Council to reimpose nuclear related sanctions.

Other components of the JCPOA are also foundering. Before the agreement, Iran was building a heavy water research reactor in Arak that would accumulate several kilograms of plutonium a year in spent fuel—enough for one or two bombs. The deal mandated a redesign of the Arak reactor to sharply curtail generation of plutonium. Even after walking away from the JCPOA,

An enriching pursuit

Iran has revved up uranium enrichment, decreasing the time needed to produce a bomb's worth of uranium-235.



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the United States had supported the redesign by waiving sanctions on other countries taking part in the work—until May, when the State Department decided to let the waivers expire as of 27 July. The move perplexed observers. "I cannot stress enough how bizarre it is to me that we demanded that the Iranians convert the reactor—and now we insist they must not convert it," says Jeffrey Lewis of the Middlebury Institute of International Studies at Monterey.

If the Arak redesign falls through, Iran could claim that Western powers are out of compliance with the JCPOA, the diplomat says. Work will continue at Arak, which Iran has renamed Khondab, says Ali Akbar Salehi, president of the Atomic Energy Organization of Iran. "Soon the international community will witness our new achievements at the Khondab Research Reactor," Salehi told *Science*. "Although sanctions impose some constraints, it invigorates us." He did not provide details, and analysts are not sure what Iran's plans are for the reactor.

U.S. withdrawal from the JCPOA has also scuttled a grand plan to turn an underground facility near the holy city of Qom into an international research center. Under the deal, Iran mothballed 700 uranium enrichment centrifuges at the Fordow site and worked with Russia to convert 328 others to producing isotopes for medicine. JCPOA negotiators floated other ideas, including installing a particle accelerator in the cramped space. But late last year, Iran resumed uranium enrichment in one Fordow hall. "That created a bit of a conundrum," says Johnson, who was involved in the deal's implementation during the Obama administration. Even minute traces of uranium would contaminate the medical isotope centrifuges. Worsening matters, a few weeks later, the United States canceled a sanctions waiver for the medical isotope work, prompting Russia to back away. Iran will continue to operate a dozen Russian-modified centrifuges on its own.

The failure to convert Fordow to a civilian research center "is a missed opportunity," says Andrea Stricker, a nonproliferation analyst at the nonprofit Foundation for Defense of Democracies.

Despite the nuclear deal's slow-motion collapse, observers don't expect Iran to open up the throttle on its program—at least not before the U.S. elections in November. If Democratic presidential candidate Joe Biden wins, the next administration "may try to resurrect some form of the JCPOA," Stricker says. "And the Iranians would probably want to test and see what they can get." ■

Richard Stone is senior science editor at the Howard Hughes Medical Institute's Tangled Bank Studios.

SCIENTIFIC COMMUNITY

NIH requires disclosure about sexual harassment by grantees

Changes aimed in part at making sure institutions don't "pass the harasser"

By **Jocelyn Kaiser**

The National Institutes of Health (NIH) is tightening grant rules to demand institutions tell it about sexual harassment by researchers it supports. Starting 12 June for new awards, NIH will expect institutions it funds to report when an investigator is removed from a grant because of harassment findings or allegations. NIH also wants to know when an investigator moves their grant to another institution because of sexual misconduct issues.

Director Francis Collins and other officials announced the new reporting guidance in an online editorial in *Science* last week. Together, the requirements will "close two important gaps" in the agency's policies, says NIH Associate Director for Science Policy Carrie Wolinetz, and should prevent cases in which institutions "pass the harasser" without the agency's knowledge. The changes will "further foster a culture whereby sexual harassment and other inappropriate behaviors are not tolerated," NIH officials write.

> **"It's definitely a step in the right direction."**
> **Carol Greider**, Johns Hopkins University

The changes align NIH's policies more closely with those of the National Science Foundation (NSF), although a gap remains. NSF requires institutions to notify the agency within 10 days of sexual misconduct findings—even if there's no change in an investigator's grant status. NIH has said that because it is not an independent agency like NSF, it doesn't have the legal authority to adopt that policy without lengthy formal rulemaking.

But NIH does have authority to ask about changes in grant personnel. Institutions must seek NIH approval for such changes, which can be due to factors including medical leave, job changes, or misconduct investigations. Until now, NIH did not ask the reasons. But the agency recently realized it could fold harassment into grant rules that require "safe and healthful working conditions," Wolinetz says. An 11 June notice says any request to NIH to change the grant's key personnel should "mention as to whether change(s) ... is related to concerns about safety and/or work environments (e.g. due to concerns about harassment, bullying, retaliation, or hostile working conditions)."

If an institution reports that a grant's personnel changed because of allegations or findings of harassment, NIH staff will review the information and decide, for example, whether the investigator can keep the grant under supervision, or the grant should end or be transferred. If a grantee is moving to a new institution because of harassment concerns, the agency plans to inform the new institution about the investigator's record.

The changes "will hopefully capture the vast majority of cases," Wolinetz says.

"I'm very pleased. It's definitely a step in the right direction," says Johns Hopkins University biologist and Nobel laureate Carol Greider, a member of a working group of NIH's Advisory Committee to the Director (ACD) that in December 2019 advised NIH to make the policy changes. But Greider worries about loopholes.

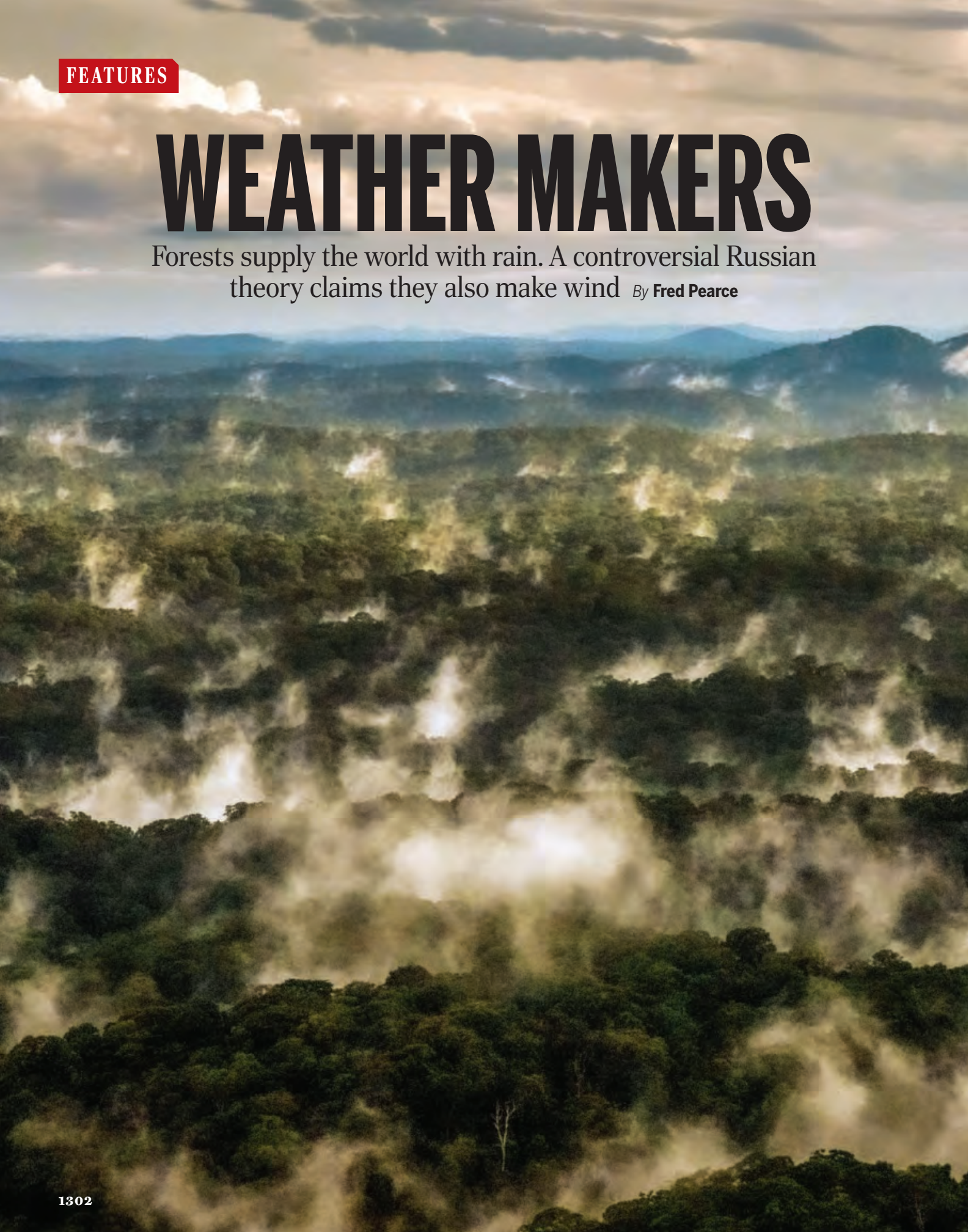
Contrary to NSF's rules, for example, institutions won't have to report findings of sexual harassment by an investigator to NIH if the investigator's status on a grant is unchanged—a step that was recommended by the working group. And such situations appear to be "a worrisome trend," Wolinetz noted last week at an ACD meeting. The growing number of sexual harassment cases voluntarily reported to NIH has made it clear that universities sometimes remove an investigator from training and supervisory duties without transferring their research grants. It seems that "Institutions are protecting their rainmakers," Wolinetz said.

Francis Cuss, a retired Bristol Myers Squibb official who co-chaired the working group, suggested NIH's policies don't go far enough and "may need ... more teeth."

Columbia University virologist Angela Rasmussen, another working group member, agrees. The new policy "is a necessary first step in a much longer journey," she says. ■

WEATHER MAKERS

Forests supply the world with rain. A controversial Russian theory claims they also make wind *By* **Fred Pearce**



Every summer, as the days get long, Anastassia Makarieva leaves her lab in St. Petersburg for a vacation in the vast forests of northern Russia. The nuclear physicist camps on the shores of the White Sea, amid spruce and pine, and kayaks along the region's wide rivers, taking notes on nature and the weather. "The forests are a big part of my inner life," she says. In the 25 years she has made her annual pilgrimage north, they have become a big part of her professional life, too.

For more than a decade, Makarieva has championed a theory, developed with Victor Gorshkov, her mentor and colleague at the Petersburg Nuclear Physics Institute (PNPI), on how Russia's boreal forests, the largest expanse of trees on Earth, regulate the climate of northern Asia. It is simple physics with far-reaching consequences, describing how water vapor exhaled by trees drives winds: winds that cross the continent, taking moist air from Europe, through Siberia, and on into Mongolia and China; winds that deliver rains that keep the giant rivers of eastern Siberia flowing; winds that water China's northern plain, the breadbasket of the most populous nation on Earth.

With their ability to soak up carbon dioxide and breathe out oxygen, the world's great forests are often referred to as the planet's lungs. But Makarieva and Gorshkov, who died last year, say they are its beating heart, too. "Forests are complex self-sustaining rainmaking systems, and the major driver of atmospheric circulation on Earth," Makarieva says. They recycle vast amounts of moisture into the air and, in the process, also whip up winds that pump that water around the world. The first part of that idea—forests as rainmakers—originated with other scientists and is increasingly appreciated by water resource managers in a world of rampant deforestation. But the second part, a theory Makarieva calls the biotic pump, is far more controversial.

The theoretical foundation of the work has been published, albeit in lesser known journals, and Makarieva has received support from a small coterie of colleagues. But the biotic pump has faced a head wind of criticism, especially from climate modelers, some of whom say its effects are negligible and dismiss the idea completely. The dispute has made Makarieva an outsider: a theoretical physicist in a world of modelers, a Russian in a field led by Western scientists, and a woman in a field dominated by men.

Half of the Amazon's rain comes from the forest's own moisture. Could it also make winds that ferry rain across continents?

Yet, if correct, the idea could help explain why, despite their distance from the oceans, the remote interiors of forested continents receive as much rain as the coasts—and why the interiors of unforested continents tend to be arid. It also implies that forests from the Russian taiga to the Amazon rainforest don't just grow where the weather is right. They also make the weather. "All I have learned so far suggests to me that the biotic pump is correct," says Douglas Sheil, a forest ecologist at the Norwegian University of Life Sciences. With the future of the world's forests in doubt, "Even if we thought the theory had only a small chance of being true, it would be profoundly important to know one way or the other."

Anastassia Makarieva and Victor Gorshkov developed the biotic pump theory at a nuclear physics institute.

PHOTOS: ANASTASSIA MAKARIEVA; (OPPOSITE PAGE) BRUSINI AURÉLIEN/HEMIS/ALAMY STOCK PHOTO

Many meteorology textbooks still teach a caricature of the water cycle, with ocean evaporation responsible for most of the atmospheric moisture that condenses in clouds and falls as rain. The picture ignores the role of vegetation and, in particular, trees, which act like giant water fountains. Their roots capture water from the soil for photosynthesis, and microscopic pores in leaves release unused water as vapor into the air. The process, the arboreal equivalent of sweating, is known as transpiration. In this way, a single mature tree can release hundreds of liters of water a day. With its foliage offering abundant surface area for the exchange, a forest can often deliver more moisture to the air than evaporation from a water body of the same size.

The importance of this recycled moisture for nourishing rains was largely disregarded until 1979, when Brazilian meteorologist Eneas Salati reported studies of the isotopic composition of rainwater sampled from the Amazon Basin. Water recycled by transpiration contains more molecules with the heavy oxygen-18 isotope than water evaporated from the ocean. Salati used this fact to show that half of the rainfall over the Amazon came from the transpiration of the forest itself.

By this time, meteorologists were tracking an atmospheric jet above the forest, at a height of about 1.5 kilometers. Known as the South American Low-Level Jet, the winds blow east to west across the Amazon, about as fast as a racing bike, before the Andes Mountains divert them south. Salati and others surmised the jet carried much of the transpired moisture, and dubbed it a "flying river." The Amazon flying river is now reckoned to carry as much water as the giant terrestrial river below it, says Antonio Nobre, a climate researcher at Brazil's National Institute for Space Research.

For some years, flying rivers were thought to be limited to the Amazon. In the 1990s, Hubert Savenije, a hydrologist at the Delft University of Technology, began to study moisture recycling in West Africa. Using a hydrological model based on weather data, he found that, as one moved inland from the coast, the proportion of the rainfall that came from forests grew,

reaching 90% in the interior. The finding helped explain why the interior Sahel region became dryer as coastal forests disappeared over the past half-century.

One of Savenije's students, Ruud van der Ent, took the idea further, creating a global model of airborne moisture flow. He combined observational data on rainfall, humidity, wind speed, and temperature with theoretical estimates of evaporation and transpiration to create the first model of moisture flow at scales larger than river basins.

In 2010, van der Ent and his colleagues reported the model's conclusion: Globally, 40% of all precipitation comes from the land rather than the ocean. Often it is more. The Amazon's flying river provides 70% of the rain falling in the Río de la Plata Basin, which stretches across southeastern South America. Van der Ent was most surprised to find that China gets 80% of its water from the west, mostly Atlantic moisture recycled by the boreal forests of Scandinavia and Russia. The journey involves several stages—cycles of transpiration followed by downwind rain and subsequent transpiration—and takes 6 months or more. "It contradicted previous knowledge that you learn in high school," he says. "China is next to an ocean, the Pacific, yet most of its rainfall is moisture recycled from land far to the west."

IF MAKARIEVA IS CORRECT, the forests supply not just the moisture, but the winds that carry it.

For a quarter-century, she worked with Gorshkov, initially as his pupil, at PNPI—part of Russia's foremost civil and military nuclear research agency, the Kurchatov Institute. They were mavericks from the start, studying ecology in a place full of physicists who use neutron beams from nuclear reactors to study materials. As theorists, she says, they had "exceptional freedom of research and thought," pursuing atmospheric physics wherever it took them. "Victor taught me: Do not be afraid of anything," she says.

In 2007, in *Hydrology and Earth System Sciences*, they first outlined their vision for the biotic pump. It was provocative from the outset because it contradicted a longstanding tenet of meteorology: that winds are driven largely by the differential heating of the atmosphere. When warm air rises, it lowers the air pressure below it, in effect creating space at the surface into which air moves. In summer, for example, land surfaces tend to heat faster and draw in moist breezes from the cooler ocean.

Makarieva and Gorshkov argued that a second process can sometimes dominate. When water vapor from forests condenses into clouds, a gas becomes a liquid that occupies less volume. That reduces air pressure, and draws in air horizontally from areas with less condensation. In practice, it means condensation above coastal forests turbocharges sea breezes, sucking moist air inland where it will eventually condense and fall as rain. If the forests continue inland, the cycle can continue, maintaining moist winds for thousands of kilometers.

The theory inverts traditional thinking: It is not atmospheric circulation that drives the hydrological cycle, but the hydrological cycle that drives the mass circulation of air.

Sheil, who became a supporter of the theory more than a decade ago, thinks of it as an embellishment of the flying river idea. "They are not mutually exclusive," he says. "The pump offers an explanation of the power of the rivers." He says the biotic pump could explain the "cold Amazon paradox." From January to June, when the Amazon Basin is colder than the ocean, strong winds blow from the Atlantic to the Amazon—the opposite of what would be expected if they resulted from differential heating. Nobre, another early acolyte, enthuses: "They don't start with data, they start with first principles."

> **"We need new international hydrological agreements to maintain the forests of source regions."**
>
> **Lan Wang-Erlandsson**, Stockholm University

Even those who doubt the theory agree that forest loss can have far-reaching climatic consequences. Many scientists have argued that deforestation thousands of years ago was to blame for desertification in the Australian Outback and West Africa. The fear is that future deforestation could dry up other regions, for example, tipping parts of the Amazon rainforest to savanna. Agricultural regions of China, the African Sahel, and the Argentine Pampas are also at risk, says Patrick Keys, an atmospheric chemist at Colorado State University, Fort Collins.

In 2018, Keys and his colleagues used a model, similar to van der Ent's, to track the sources of rainfall for 29 global megacities. He found that 19 were highly dependent on distant forests for much of their water supply, including Karachi, Pakistan; Wuhan and Shanghai, China; and New Delhi and Kolkata, India. "Even small changes in precipitation arising from upwind land-use change could have big impacts on the fragility of urban water supplies," he says.

Some modeling even suggests that by removing a moisture source, deforestation could alter weather patterns beyond the paths of flying rivers. Just as El Niño, a shift in currents and winds in the tropical Pacific Ocean, is known to influence weather in faraway places through "teleconnections," so, too, could Amazon deforestation diminish rainfall in the U.S. Midwest and snowpack in the Sierra Nevada, says Roni Avissar, a climatologist at the University of Miami who has modeled such teleconnections. Far-fetched? "Not at all," he says. "We know El Niño can do this, because unlike deforestation, it recurs and we can see the pattern. Both are caused by small changes in temperature and moisture that project into the atmosphere."

Lan Wang-Erlandsson, who researches interactions between land, water, and climate at Stockholm University, says it's time for water resource managers to shift their focus from water and land use within a river basin to land-use changes occurring outside it. "We need new international hydrological agreements to maintain the forests of source regions," she says.

Two years ago, at a meeting of the United Nations Forum on Forests, a high-level policy group on which all governments sit, David Ellison, a land researcher at the University of Bern, presented a case in point: a study showing that as much as 40% of the total rainfall in the Ethiopian highlands, the main source of the Nile, is provided by moisture recycled from the forests of the Congo Basin. Egypt, Sudan, and Ethiopia are negotiating a long-overdue deal on sharing the waters of the Nile. But such an agreement would be worthless if deforestation in the Congo Basin, far from those three nations, dries up the moisture source, Ellison suggested. "Interactions between forests and water have been almost entirely ignored in the management of global freshwater resources."

The biotic pump would raise the stakes even further, with its suggestion that forest loss alters not just moisture sources, but also wind patterns. The theory, if correct, would have "crucial implications for planetary air circulation patterns," Ellison warns, especially those that take moist air inland to continental interiors.

THE THEORY'S SUPPORTERS are a minority. In 2010, Makarieva, Gorshkov, Sheil, Nobre, and Bai-Lian Li, an ecologist at the University of California, Riverside, submitted what was meant to be a landmark description of the biotic pump to *Atmospheric Chemistry and Physics*, a major journal with open peer review. Titled "Where Do Winds Come From?" the paper faced a barrage of criticism online, and it took the journal many months to find two scientists willing to review it. Isaac Held, a meteorologist at Princeton University's Geophysical Fluid Dynamics Laboratory, finally volunteered—and rec-

ommended rejection. "This is not a mysterious effect," he says. "It is small and included in some atmospheric models." Critics said the expansion of air from heat released when water vapor condenses counteracts the space-creating effect of condensation. But Makarieva says the two effects are spatially separate, with the warming effect happening aloft, and the pressure drop of condensation occurring closer to the surface, where it generates the biotic wind.

The other reviewer was Judith Curry, then an atmospheric physicist at the Georgia Institute of Technology, who has long had concerns about the atmospheric dynamics at the core of climate models. She felt it was important to publish the paper and says the standoff was "very bad for climate science, which badly needs an infusion from hardcore physicists." After 3 years of debate, the journal's editor overruled Held's recommendation and published the paper, saying it was published "not as an endorsement" but "to promote continuation of the scientific dialogue on the controversial theory [that] may lead to disproof or validation."

Since then, there has been neither validation nor disproof, but largely a standoff. Gavin Schmidt, a climate modeler at Columbia University, says, "It's simply nonsense." The authors' responses to criticisms were "really just mathematics that gave no one any confidence that there was any point in continuing the dialogue." Jose Marengo, a meteorologist in Brazil and head of the National Centre for Monitoring and Warning of Natural Disasters, says: "I think the pump exists, but it's very theoretical right now. The climate model community hasn't embraced it, but the Russians are the best theoreticians in the world, so we need proper field experiments to test it." Yet no one, including Makarieva, has yet proposed clearly what such a test might look like.

For her part, Makarieva is building on the theory, arguing in a series of recent papers that the same mechanism can affect tropical cyclones, which are driven by the heat released when moisture condenses over the ocean. In a 2017 paper in *Atmospheric Research*, she and her colleagues proposed that biotic pumps set up by the forests on land draw moisture-rich air away from the cyclone nurseries. This, she says, might explain why cyclones rarely form in the South Atlantic Ocean: The Amazon and Congo rainforests between them draw so much moisture away that there is too little left to fuel hurricanes.

Kerry Emanuel, a leading hurricane researcher at the Massachusetts Institute of Technology, says the proposed effects "while not negligible are very small." He prefers other explanations for the lack of South Atlantic hurricanes, such as the region's cool waters, which send less moisture into the air, and its strong shearing winds, which disrupt cyclone formation. Makarieva is equally dismissive of the traditionalists, saying some of the existing theories for hurricane intensity "conflict with the laws of thermodynamics." She has another paper on the topic under peer review at the *Journal of the Atmospheric Sciences*. "We are concerned that, despite the editor's encouragement, our work will get rejected once again," she says.

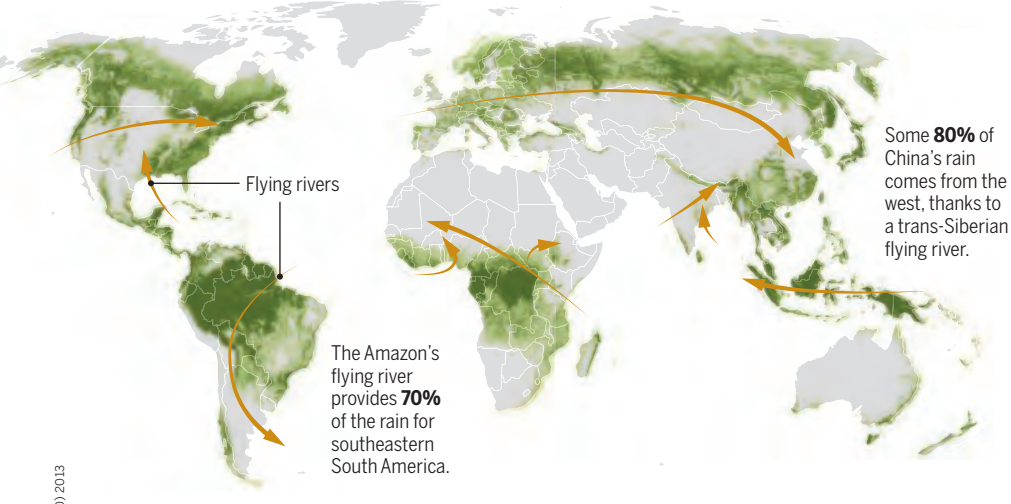
Even if Makarieva's ideas are fringy in the West, they are taking root in Russia. Last year, the government began a public dialogue to revise its forestry laws. Aside from strictly protected areas, Russian forests are open to commercial exploitation, but the government and the Federal Forestry Agency are considering a new designation of "climate protection forests." "Some representatives of our forest department got impressed by the biotic pump and want to introduce a new category," she says. The idea has the backing of the Russian Academy of Sciences. Being part of a consensus rather than the perennial outsider marks a change, Makarieva says.

This summer, the coronavirus lockdown put the kibosh on her annual trip to the northern forests. Back in St. Petersburg, she has settled down to respond to yet another round of objections to her work from anonymous peer reviewers. She insists the pump theory will ultimately prevail. "There is a natural inertia in science," she says. With a dark Russian humor, she invokes the words of the legendary German physicist Max Planck, who is said to have once remarked that science "advances one funeral at a time." ■

Fred Pearce is a journalist in London.

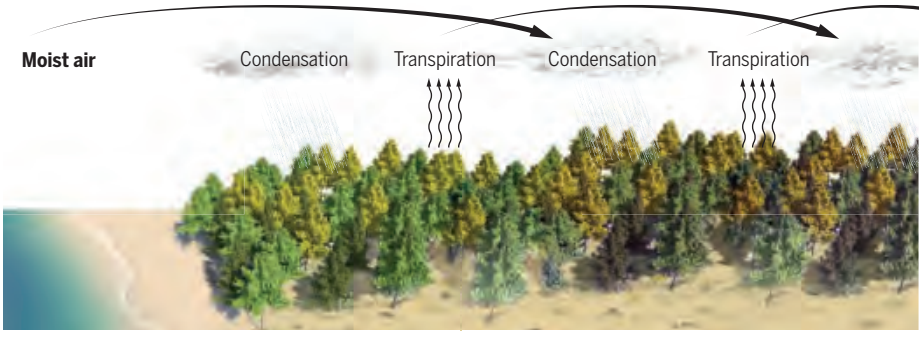
Rain parades

So-called flying rivers are prevailing winds that pick up water vapor exhaled by forests and deliver rains to distant water basins. A controversial theory suggests forests themselves drive the winds (bottom).


Flying rivers
The Amazon's flying river provides **70%** of the rain for southeastern South America.
Some **80%** of China's rain comes from the west, thanks to a trans-Siberian flying river.

Sowing the wind

The biotic pump theory suggests forests not only make rain, but also wind. When water vapor over coastal forests condenses, it lowers air pressures, creating winds that draw in moist ocean air. Cycles of transpiration and condensation can set up winds that deliver rains thousands of kilometers inland.



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EVOLUTION

The origins of flowering plants and pollinators

New research raises questions about when flowering plants and their pollinators evolved

By **Casper J. van der Kooi**[1] *and* **Jeff Ollerton**[2]

For more than a century there has been a fascination with the surprisingly rapid rise and early diversity of flowering plants (angiosperms). Darwin described the seemingly explosive diversification of angiosperms as an "abominable mystery," and debates continue about the origin and processes driving angiosperm speciation. Dating the origin of angiosperms was traditionally the prerogative of paleobotanists who read the fossil record of plants, but with DNA sequencing becoming increasingly sophisticated, molecular dating methods have come to the table. Many angiosperm fossils can be dated to the Early Cretaceous (~135 million years ago), which has led paleobotanists to reason that they originated during that era. It is now increasingly recognized that angiosperms are probably older than the oldest fossils, but how much older remains controversial. When angiosperms originated is key to understanding the origin and evolu-

PHOTO: JONATHAN BLAIR/NATIONAL GEOGRAPHIC IMAGE COLLECTION

The aquatic angiosperm *Archaefructus liaoningensis* is one of the earliest fossil angiosperms to have been identified so far.

tion of pollinators, particularly insects such as bees, butterflies, moths, and flies.

Recent reports highlight the disparity of molecular and paleontological time scales and draw conflicting conclusions about the timing of angiosperm diversification (see the figure). On the basis of gene sequences from 2881 chloroplast genomes belonging to species from 85% of living flowering-plant families, time-calibrated using 62 fossils, one study (*1*) dated the origin of angiosperms to the Late Triassic, >200 million years ago. This is ~70 million years (roughly the equivalent of the Jurassic) before the earliest accepted angiosperm fossils. This study further suggests that major radiations (species diversification) occurred in the Late Jurassic and Early Cretaceous, ~165 to 100 million years ago. By contrast, an overview of paleobotanical evidence (*2*) refutes a substantive pre-Cretaceous diversification, with only some specific clades (such as water lilies) perhaps originating during the Late Jurassic. The sequential appearance of different types of fossils and morphological characteristics is proposed to render major earlier diversification events unlikely, supporting previous studies (*3*, *4*). Although the idea that angiosperms arose around the beginning of the Cretaceous may seem hard to reconcile with the rapid increase in morphological diversity observed during that interval, it is not impossible if the Cretaceous radiation occurred rapidly.

Both paleontological records and molecular analyses have their strengths and weaknesses. The strength of fossils is that they can provide information on past form, function, and clade richness, and indirectly provide information on speciation and extinction. Fossils are particularly useful when they harbor intermediate structures or combinations of characters that no longer exist, which can provide insightful examples that help to reconstruct the course of evolutionary events. However, the interpretation of fossils can be subjective and controversial, because important features of these plants may not be preserved and often must be inferred from two-dimensional compressed remains.

The absence of evidence is no evidence of absence, and it is known that the fossil record can be incomplete or biased because some taxa may be less likely to fossilize. For example, specific ecologies or habitats will influence the likelihood of whole-plant fossilization, although pollen is a useful exception because it can generally survive more extreme conditions. Furthermore, anchoring a fossil to a specific time period relies on accurately dating the stratum in which it was found, which can also be problematic, although the error margin caused by this factor is usually small. It is important to keep in mind that there can be a considerable lag between time of origin and the earliest recognizable fossil, because fossils generally appear when a taxon has existed for some time and in relatively high frequencies, a phenomenon known as the Signor-Lipps effect.

Molecular analyses are built on hard-to-estimate variables, such as the distribution of mutation rates across taxa and time. Variation in divergence times—which inevitably occurs in datasets with many species—frequently leads to overestimation of age (*5*, *6*). Indeed, molecular analyses often push origin dates back in time, including the older lineages, but whether this is a methodological error remains unclear.

One of the hallmarks of angiosperms is their relationship with animal pollinators, especially insects. As with plants, the diversification of insects is a field with many uncertainties. The origin of several important orders of flower-visiting insects (e.g., Coleoptera, Diptera, Hymenoptera, and Lepidoptera) lies in the Permian or Triassic (300 to 200 million years ago) with marked periods of diversification in the Cretaceous, which is frequently mentioned to coincide with the main angiosperm radiation (*7*). However, the timing of the origin of flower-visiting insects is debated. For example, for Lepidoptera (butterflies and moths), a Late Triassic radiation has been suggested on the basis of fossil evidence (*8*), but a recent study using transcriptomes covering nearly all Lepidoptera superfamilies dated the origin even further back, during the Carboniferous (~300 million years ago) (*9*). Although butterfly diversification may be triggered more by host plant chemistry than by floral diversity—which need not be correlated—given the importance of butterflies and moths for angiosperm reproduction, their diversification is important in understanding plant-pollinator interactions.

Notwithstanding that the timing of the origin of angiosperms remains debated, if angiosperms arose before the Jurassic, this has profound implications for understanding how insect pollination evolved. There is little doubt that insect pollination accelerated the angiosperm radiation; however, which factor triggered what evolutionary event becomes more complex given the latest findings. It was long considered that wind pollination in early-diverging nonflowering seed plants (gymnosperms) was replaced by animal pollination in angiosperms, and that this switch to animal pollination led to angiosperm diversification, but this seems an oversimplification (*10*).

Many now-extinct gymnosperms (e.g., Bennettitales) were insect pollinated, and angiosperms could have evolved either directly from insect-pollinated gymnosperms or from wind-pollinated gymnosperms in such a way that they co-opted insects that were servicing gymnosperms in the same community. Conversely, if the earlier Triassic origin of angiosperms is correct, some gymnosperms may have co-opted insects as pollinators from early angiosperms. It seems unlikely, however, that this latter process was important in

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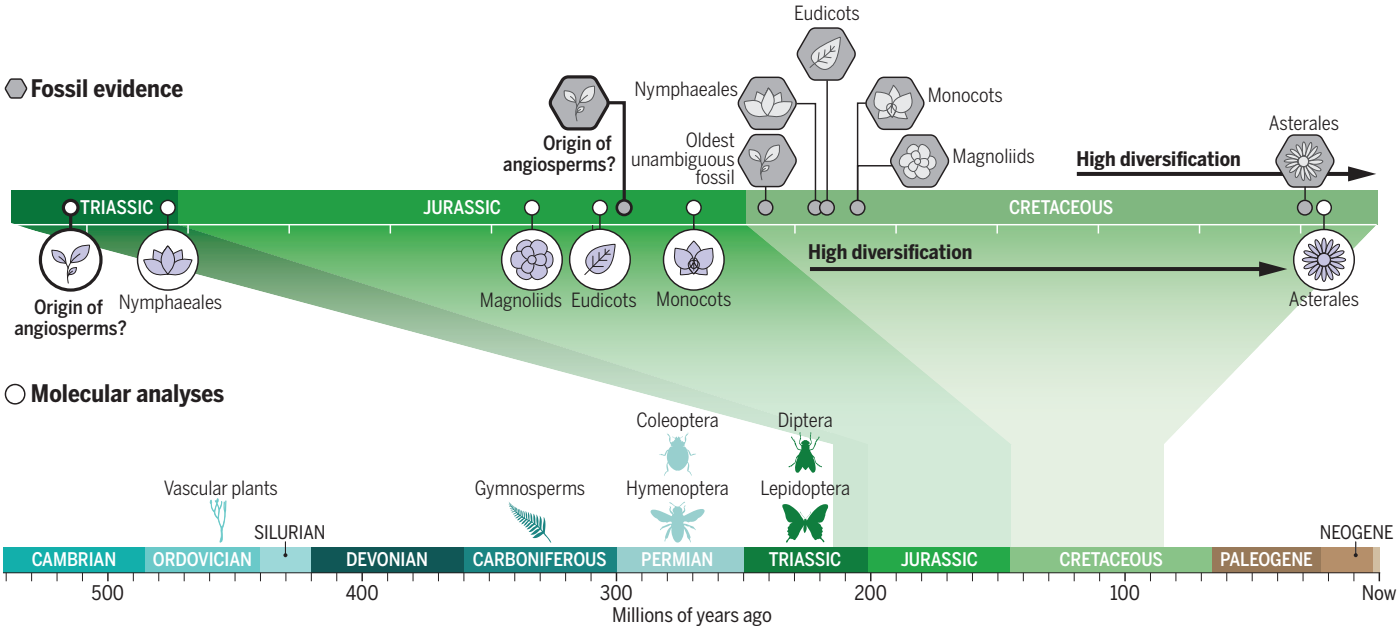
Evolution of angiosperms according to molecular and fossil evidence

Fossil and molecular evidence lead to conflicting conclusions about the timing of the origin of flowering plants. Fossil evidence suggests that flowering plants arose near the beginning of the Cretaceous, but molecular analyses date the origin much earlier, in the Triassic.



the scheme of angiosperm evolution, because even if they occurred at this earlier period, angiosperms were not a dominant plant group in the Jurassic. By contrast, Bennettitales and other early seed plants were ecologically dominant in Late Triassic to Jurassic floras, indicating that the transition to insect pollination in angiosperms arose through these gymnosperm groups. These possibilities are more complex than the standard scenarios that envisioned a progression from primitive wind pollination to advanced insect pollination. They hint at a richer ecological milieu of more complex interactions between species than had previously been appreciated, including insect groups that are currently much less important as pollinators, such as scorpionflies (Mecoptera) (*11*).

The timing of flowering-plant origins also provides a minimum age for the evolution of their most prominent feature: flowers. Insect pollination in many extant gymnosperms (e.g., cycads, *Ephedra*, *Gnetum*) is facilitated mainly by scent rather than by visual attraction. The same may have been true of the extinct gymnosperms, but because scent does not fossilize, it may be impossible to ever know. However, if the reproductive structures of these extinct gymnosperms functioned in a manner similar to their living relatives, with odor predominating, then the increasing importance of visual-based cues to attract pollinators in angiosperms could be one of the defining features of angiosperm evolution and success. Further, if floral structures predate some speciose orders of flower-visiting insects, perhaps flower features have shaped trait evolution in these large insect groups.

There are clear examples of coevolution of specific floral and pollinator morphological characteristics in some systems, such as floral tube length and pollinator tongue length (*12*). What about floral features such as color and scent? For example, perhaps floral color and scent evolved to match pollinator vision and olfaction, or vice versa. Alternatively, signal production may have evolved synchronously with detection. The basic principles of color vision in insects, such as the possession of three types of photoreceptors (ultraviolet, blue, green), seem to predate flowers regardless of whether they arose during the Triassic or later (*13*). Because color vision is also used for key behaviors such as detecting potential mates and predators and finding oviposition (egg-laying) sites, the evolution of color vision is unlikely to be driven by flower colors.

A similar ancestral origin of olfaction compared to scent production was documented in a group of plants pollinated by scarab beetles (*14*), where odor reception by pollinators predates production of the scent signal by plants. However, behavioral aspects of olfaction or color vision, such as innate color preferences that shape foraging behavior in various insect groups (*15*), may have evolved later, in response to floral signals. All of this depends on the timing of the evolution of flowering plants as well as the order of evolutionary events that led to insect pollination. If insect-pollinated gymnosperms predate angiosperms, for example, then it may be possible to trace the origin of these visual and olfactory traits to long-extinct clades of plants that once dominated terrestrial floras.

Future paleontological discoveries will undoubtedly reveal additional fossils, and the use of complementary sequencing approaches and more sophisticated evolutionary models will help to mitigate the limitations imposed by the rampant polyploidy in plants that frequently hinders analysis of nuclear genes. Whether Darwin's question about the timing of flowering-plant evolution and radiation will ever be answered remains a mystery, but clearly this question and its ecological implications for understanding insect pollination are complicated. ■

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PHOTOVOLTAICS

Perovskite solar cells take a step forward

A new encapsulation technique helps move a photovoltaic toward commercialization

By **Emilio J. Juarez-Perez**[1,2,3] and **Marta Haro**[3]

Today's monocrystalline silicon solar cells have their throne on the roofs of our houses. In the past decade, however, perovskite solar cells (PSCs) show impressive advances with a high power conversion efficiency (PCE) of 25.2% (*1*) and low fabrication cost, which make this technology promising for further advances in decarbonization energy models (*2*). Yet the life cycle of PSCs needs to be increased for market integration. Poor stability is the main impediment to commercializing this technology. Thus, great effort has been focused on the causes and mechanisms of degradation, many of which can be mitigated or minimized with encapsulation. Various strategies have been proposed to increase PSCs' operational stability, which is affected by moisture, oxidation, heat, light, and other factors (*3*, *4*). On page 1328 of this issue, Shi *et al.* (*5*) report a successful encapsulation procedure for hybrid PSCs.

"...vapors retained by encapsulation could regenerate perovskite during night hours..."

The standard test to assess the long-term operation of photovoltaic terrestrial flat-plate modules in open-air climates is referred to as IEC 61215:2016. The IEC 61215 standard was developed by the International Electrotechnical Commission (IEC) for working photovoltaic devices that incorporate robust inorganic light-harvesting materials such as silicon, cadmium telluride, copper indium selenide, and copper indium gallium selenide. The stability test must consider all components and encapsulating materials as a whole. Achieving a high score on a solar cell stress test has been challenging for organic-inorganic hybrid perovskite-based devices (*6*). The device developed by Shi *et al.* retained >95% of its initial efficiency after undergoing the harsh environmental conditions of the Damp Heat and Humidity Freeze tests. By doing so, their device exceeded the requirements of IEC 61215:2016.

The key strategy for the authors' success was the polymeric "blanket-cover" encapsulation that simultaneously prevented moisture/air ingress into the device and suppressed volatile organic gas release from the hybrid perovskite. The analysis of outgassing from several perovskite and precursor assemblies, monitored by coupled gas chromatography–mass spectrometry, was essential for determining and implementing the best encapsulation strategy. Beyond the simplicity of this encapsulation approach, the authors demonstrated the feasibility of developing low-cost packaging techniques for PSCs, in contrast with more technically challenging and expensive processes of forming a protective barrier, such as atomic layer deposition or chemical vapor deposition.

A reproducible high PCE (>20%) combined with the authors' demonstration that their PSC device can pass the harsh IEC 61215:2016 tests might suggest that PSCs are finally ready for commercialization. Unfortunately, each of these achievements, high efficiency and high stability, still has a downside. Deploying PCEs well above 20% is still problematic when scaling up the active area for these photovoltaics. This apparently is specific to PSCs, as it is practically nonexistent with wafer or other thin film–based photovoltaics. Moreover, the authors observed an enigmatic threshold temperature during the regular working condition for a solar cell under constant illumination, the so-called maximum power point test (MPPT). Once this temperature is exceeded, a prominent drop in efficiency initiates and drives the irreversible loss of the device. Shi *et al.* estimated this temperature threshold to be ~35° to 40°C despite their devices passing the IEC tests, which may require further specific thermal stability studies on PSCs.

This temperature threshold has been noted in a previous MPPT that successfully tested PSCs for 4000 hours of continuous illumination (*7*). Surprisingly, the threshold temperature for the earlier test was also ~40°C, even though nonmixed or pure methylammonium (MA) cation-type perovskite was used. This is in contrast to the mixed FAMA and CsFAMA perovskites [containing MA, FA (formamidinium), and cesium] used by Shi *et al.* A much higher temperature threshold would be expected for FAMA and CsFAMA because of their higher FA content. This analogous temperature threshold could be due to degradation factors such as MA content, diiodine sublimation, or disruptive ion migration. Determining this threshold in MA-free perovskites could provide insight, along with determining whether full inorganic halide perovskite-based devices are free of this threshold at MPPT conditions under 1-sun illumination. Standard stability tests are designed to stress all plausible weak points of the device and offer useful data for interlaboratory comparisons. Although considerable efforts to design specific stability stress tests for hybrid perovskites are under way (*8*, *9*), there is a lack of standardization in testing procedures, including specific active control of temperature during MPPT to find the fatal threshold temperature of the device.

Shi *et al.* provide further insight into the challenge of long-term stabilization for PSC devices by reporting the outgassing vapors generated above the ~35° to 40°C temperature threshold. The outgassing vapors retained by encapsulation could regenerate perovskite during night hours when the device cools down, but this is not possible if these vapors react irreversibly with other components of the device, such as selective contacts and metal electrodes. The chemistry of halide perovskite is quite different from the classic robust inorganic crystalline materials prepared at high temperature and used in commercial photovoltaics. Perovskite is an organic-inorganic hybrid semiconductor material, and this hybrid character can be assumed to be the clue to developing long-lasting PSCs for commercialization. ■

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INFECTIOUS DISEASES

Dating the emergence of human pathogens

Ancient genomes can narrow the search for the sources of zoonotic transmissions

By **Simon Y. W. Ho**[1] and **Sebastián Duchêne**[2]

Understanding the emergence and evolution of human pathogens plays a pivotal role in epidemiology and in predicting the trajectories of outbreaks. The application of phylogenetic methods to pathogen genomes has provided a range of insights into their evolutionary dynamics (*1*). In many cases, phylogenetic methods can use the sampling dates of the genomes to reconstruct the evolutionary time scales of viruses, bacteria, and other pathogens. Ancient genomes can increase the power of these approaches by narrowing the estimated time window of pathogen emergence and by augmenting the evolutionary temporal signal in the genetic data. On page 1367 of this issue, Düx *et al.* (*2*) show how a century-old genome of *Measles morbillivirus*, extracted from human lung tissue, can help efforts to pinpoint the time of emergence of measles.

A distinctive feature shared by many human pathogens is that their evolution can be observed over epidemiological time scales. These pathogens can undergo measurable genetic change even between successive sampling dates. The evolutionary rates of these pathogens can be estimated by analyzing their genomes while taking the sampling dates into account (*3*). Datasets that are amenable to such analysis are referred to as having "temporal signal" (*4*).

Strong temporal signals are often detected in analyses of rapidly evolving RNA viruses. However, they can also be detected in genomic data from more slowly evolving bacteria, as demonstrated in studies of the tuberculosis pathogen *Mycobacterium tuberculosis* (*5*) and *Salmonella enterica* (*6*). Conversely, temporal signals have proven to be rather more elusive in some pathogens, such as the bacterium that causes leprosy, *Mycobacterium leprae* (*7*).

The temporal signal can be amplified by widening the sampling period, which is most effectively done by sequencing genomes from samples in historical collections or from archaeological remains. These efforts have been substantially aided by advances in molecular techniques and sequencing technology. However, obtaining genomic data from RNA viruses (such as the measles virus), which degrade rapidly in the environment, continues to be extremely challenging (*8*). In this regard, the sequencing of a century-old genome of the measles virus by Düx *et al.* is a profound achievement.

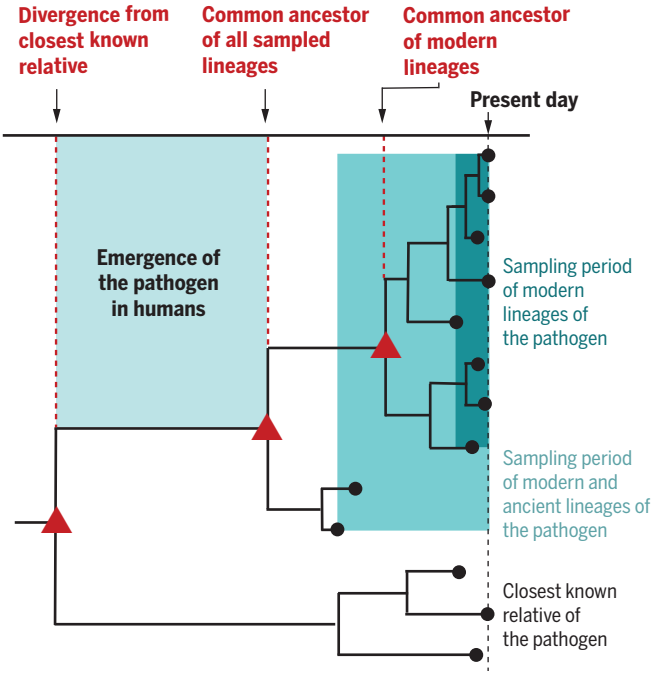
Increasing the temporal signal in the measles virus genomic dataset leads to a more accurate estimate of the evolutionary rate of the pathogen, which allows inference of the date of the common ancestor of the sampled lineages with greater confidence. This date places a minimum bound on the emergence of the pathogen in humans (see the figure), because the common ancestor implies a human-to-human transmission event.

The inclusion of ancient genomes can also push back the date of the common ancestor of the pathogen by sampling extinct lineages (*8*). In this way, ancient genomes are able to cast light on a hidden period of evolutionary history, an effect that is seen in the analysis of the measles virus by Düx *et al.* and in previous studies of the bacterium *S. enterica* (*6*), smallpox (variola) virus (*9*), and the plague bacterium *Yersinia pestis* (*10*). Sampling extinct lineages is particularly important for pathogens that have undergone recent reductions in genetic diversity. These include viruses that have been subject to large-scale vaccination programs, such as measles (as reported by Düx *et al.*), as well as those that have been targeted by extensive treatment and prevention strategies, such as human immunodeficiency virus type 1 (HIV-1) (*11*). In these cases, there is likely to have been a substantial assortment of extinct lineages that have not left any traces in the present-day population of the pathogen.

The maximum age of a pathogen's emergence in humans is bounded by its evolutionary divergence from its closest known relative. Düx *et al.* estimated that the measles virus diverged from its closest relative, the cattle pathogen *Rinderpest morbillivirus*, more than two millennia ago. The authors then posited that the establishment of measles in humans coincided with population growth in Eurasia between 2000 and 2500 years ago, although it could have occurred much more recently. This estimate is consistent with other studies that have suggested that the rise of human settlements facilitated the spread of some infectious pathogens, including the plague bacterium (*12*).

Pinpointing the emergence of human pathogens

The emergence of human pathogens can be bracketed between their divergence from their closest relatives and the common ancestor of all sampled lineages of the pathogen. Including ancient genomes expands the sampling period and can reveal extinct lineages of the pathogen.


Divergence from closest known relative
Common ancestor of all sampled lineages
Common ancestor of modern lineages
Present day
Emergence of the pathogen in humans
Sampling period of modern lineages of the pathogen
Sampling period of modern and ancient lineages of the pathogen
Closest known relative of the pathogen

GRAPHIC: H. BISHOP/*SCIENCE*

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For pathogens with sources that are less well understood, the window of uncertainty can be narrowed by searching for close relatives of the pathogen. Such a search can be targeted at candidate reservoir hosts or can involve a broader survey of pathogen diversity across wildlife. For example, the growing application of metagenomics (nontargeted genome sequencing) approaches has led to a staggering expansion of the known diversity of viruses (*13*), while revealing that animal hosts can carry a wide range of viruses in abundance. Nevertheless, huge swathes of the virosphere remain unexplored.

Phylogenetic dating analyses of pathogens have come under the spotlight in the ongoing coronavirus disease 2019 (COVID-19) pandemic. The closest known relative of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a coronavirus from a horseshoe bat, but these two viruses diverged from each other several decades ago (*14*). The most recent common ancestor of sampled SARS-CoV-2 genomes has been dated to late November to early December 2019 (*15*). The gap between these two events has hindered attempts to identify the reservoir host of SARS-CoV-2 and the timing of the zoonotic spillover into human hosts.

Further genomic data from historical and ancient samples, along with more comprehensive and intensive surveys of viruses harbored in wildlife, will lead to continued refinements of the time scales of emergence and evolution of human pathogens. In turn, these refinements will improve our understanding of the circumstances under which pathogens emerge in their hosts and the mechanisms by which they do so. ■

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PHOTOS: NEUBARTH *ET AL.* (3)

NEUROSCIENCE

Getting a grip on touch receptors

Meissner corpuscles are anatomically complex mechanosensors critical for tactile acuity

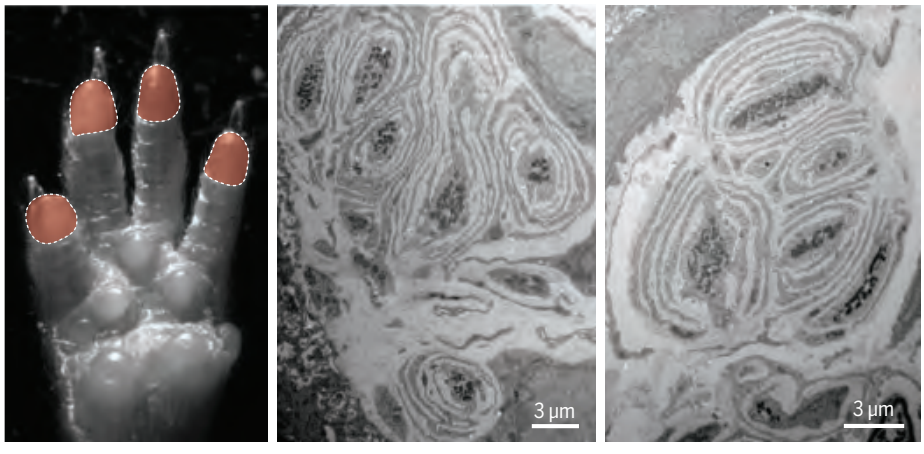
By **Kara Marshall** *and* **Ardem Patapoutian**

Skin sets the boundaries of the body, and neuron endings within the skin translate pressure into percept. When exploring a surface with our hands, exquisitely mechanosensitive touch neurons can detect tiny wrinkles in texture down to 10 nm (*1*) or mere 3° differences in edge orientation (*2*). These feats are accomplished by diverse sensory cells that are tuned to capture the subtleties of our external world. On page 1330 of this issue, Neubarth *et al.* (*3*) characterize Meissner corpuscles, mechanosensory end organs in the skin that detect touch. The authors report that in mice, two neuron types comprise a single Meissner corpuscle and that differences in their stimulus thresholds, electrical conduction velocity, and response patterns could underly the sensitivity range of Meissner corpuscles. They also demonstrate that these receptors are critical for tactile acuity.

The skin is vast. Touch receptors cover every bodily surface, and their varied morphologies point to equally diverse functions. Merkel cell–neurite complexes that detect gentle touch cup epidermal ridges in the fingertips but elsewhere terminate closer to the skin surface. Lanceolate endings encircle the base of each hair and detect hair movement. Pacinian corpuscles sit deep in the skin whereas Meissner corpuscles are superficial, but both detect vibrations (*4*). This distributed array, and the lack of specific markers, has made it difficult to study individual touch receptor types.

Meissner neuron afferents are rapidly adapting, meaning that they have brief responses to sustained pressure applied to the skin. This response profile is ideal for detecting movement and small slips that occur when scanning an object (*5*). Thus, Meissner corpuscles have been hypothesized to be critical for tactile acuity, but the only way to confirm this is to selectively remove them. Neubarth *et al.* accomplish this in the mouse. The breadth of their findings was enabled by a variety of tools, ranging from electron microscopy to naturalistic behavioral paradigms.

The morphology of neuron endings in the skin is inherently related to function. For example, hair follicles act as levers for the neurons that surround them, and ample lamellar wrappings from Schwann cells create a filter that shields Pacinian corpuscles from low-frequency vibrations. Meissner corpuscles are also wrapped in lamellae and poised to detect superficial stimuli, as they sit at the top of dermal papillae. Genetic tools to distinguish distinct touch receptor populations have not been available until recently (*6, 7*). By combining known Meissner neuron marker genes, Neubarth



Mouse digits have a high density of Meissner corpuscles (left). Electron microscopy of Meissner corpuscles reveals that TrkB-expressing neurons (middle) have more Schwann cell wrappings than Ret-expressing neurons (right).

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et al. found that two genetically distinct neurons intertwine in a single Meissner corpuscle. Electron microscopy revealed that neuron endings that express Ret (rearranged during transfection) receptors had fewer lamellar wrappings compared to neuron endings that express TrkB (tropomyosin receptor kinase B) receptors (see the figure).

Lamellar wrappings dampen vibration responses of Pacinian corpuscles, and removing lamellae converts rapidly adapting responses into sustained ones (*8*). Indeed, in vivo recordings revealed functional differences between Meissner afferent types: Compared to TrkB-expressing neurons, Ret-expressing neurons had higher thresholds, faster electrical conduction velocity, and sometimes sustained responses, but lacked "off" responses to stimulus removal. It is not clear whether these properties are conferred by the modest variations in lamellar structure, differences in protein expression, or a combination of mechanisms. The findings do suggest that a Meissner corpuscle is two sensors joined into one, which offers an intriguing mechanism by which a single receptor can achieve greater dynamic range.

What information do Meissner corpuscles convey? For years, the standard tool for studying mouse touch has been von Frey filaments, which are a set of probes with differing stiffness. The readout for whether a mouse feels these probes is paw movement when poked, but mice vary in their motivation, and unexpected pokes do not represent normal tactile experiences. Scientists have recently developed newer assays to probe touch in more creative ways. For example, mice prefer still surfaces to vibrating ones and will ceaselessly work to remove tape stuck on their back (*9*). Tasks that probe recognition of unfamiliar objects can be used for testing texture detection, and air puffs can test hair movement sensation (*10*). These developments have expanded our understanding of touch, but they fall short of psychometric tests for skill. Moreover, they offer a limited understanding of how mice use touch in natural settings.

Neubarth *et al.* designed an operant conditioning paradigm using a water reward delivered after a gentle touch, which allowed them to build psychometric curves. Mice lacking Meissner corpuscles were far less skilled at detecting gentle indentation in this task. Additionally, behavioral analysis of mice handling sunflower seeds revealed that Meissner corpuscles are critical for fine sensorimotor control. This experimental evidence connects a specific touch receptor with naturalistic tactile behavior. The deficits observed in these animals, despite having many other functional touch receptors, emphasize that Meissner corpuscles are indispensable for creating the full spectrum of touch sensation. Future work could disentangle the differential contributions of each Meissner neuron subtype to these tasks.

Rapidly adapting responses from Meissner corpuscles are ideal for conveying dynamic touch, and their sensitivity to movement and slip have long made them candidates for fine-tuning grip. Primate species that spend more time handling fruit have higher densities of Meissner corpuscles in their palm skin (*11*). The findings of Neubarth *et al.* solidify this role. Nonetheless, these receptors are insensitive to static stimuli and poorly resolve spatial details (*4*). Many studies have confirmed that spatial discrimination, particularly of coarse or static stimuli, is better served by slowly adapting responses from Merkel cell–neurite complexes (*4*). Behavioral tasks that select for spatial discrimination will be crucial to fully understand the functional contributions of different touch receptors.

These data raise several questions. Where does the mechanotransduction ion channel PIEZO2 (Piezo type mechanosensitive ion channel component 2), which is necessary for touch sensation (*9, 12*), sit in this structure? Neubarth *et al.* and others (*13*) identified small lamellar openings that expose the naked axon to the extracellular matrix. Might these openings be sites of mechanotransduction? It is also unclear what benefit dual innervation offers. Computational modeling suggests that the organization observed for Meissner corpuscles increases tactile information and acuity (*3*). This could be a broader theme among touch receptors. Different neurons are neatly interdigitated in lanceolate endings (*13*), and Merkel cells occasionally have atypical neurons wandering nearby (*14*). These neurons are not as intimately intertwined as in Meissner corpuscles, but perhaps they function similarly. These exciting findings pave the way for understanding the full complexity of touch receptor function. ■

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ORGANIC CHEMISTRY

Closing the radical gap in chemical synthesis

Unstable radical intermediates are harnessed in a microfluidic electrochemical cell

By **Jian-Quan Liu[1,2], Andrey Shatskiy[2], Markus D. Kärkäs[2]**

Chemists now face the daunting task of identifying synthetic methods that are more practical, scalable, and sustainable. Approaches include the development of safer, more readily available reactants and reagents as well as discoveries in method development that provide more effective and selective synthetic transformations (*1, 2*). Conventionally, organic synthesis has been conducted through batch processes, but the use of continuous-flow technologies can allow straightforward scale-up, facilitate operational simplicity, and reduce occupational hazards and industrial waste streams (*3*). On page 1352 of this issue, Mo *et al.* (*4*) demonstrate that microfluidic electrochemical technologies can be applied to single-electron transfer (SET) redox-neutral reactions. Their strategy enables the efficient formation and utilization of free-radical intermediates by placing the anode and cathode in close proximity to overcome radical instability. These versatile intermediates were leveraged in Minisci-type reactions of electron-deficient heterocyclic compounds, as well as radical-radical and nickel-catalyzed sp^2 carbon–oxygen cross-couplings.

Students of organic chemistry are mainly taught the idea of assembling organic compounds by reacting a pair of oppositely charged species, such as electrophiles with nucleophiles (*5*). However, these ionic transformations often have considerable activation barriers that must be overcome

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thermally. Alternatively, lower-energy pathways can be accessed through the use of catalysis or additives. By contrast, many emerging approaches rely on free-radical chemistry. In organic synthesis, radical reactions historically have been perceived as uncontrollable and impractical, but this perception has gradually shifted, mainly because of the conceptual advances in the areas of photoredox catalysis (*6, 7*) and electrosynthesis (*8, 9*).

Redox-modulating techniques allow chemists to target functional groups in a molecule on the basis of their different redox potentials, thereby enabling the mild and selective generation of radicals. Compared to conventional two-electron chemistry, using radicals opens up alternative reactivity and selectivity profiles and can greatly simplify synthesis through non-traditional bond construction. The revitalized interest in radicals has propelled their incorporation in contemporary chemical synthesis (*10, 11*).

Reactions that proceed through radical intermediates can be classified as net oxidations, net reductions, or redox neutral. In the first two cases, stoichiometric reagents must be used as electron donors or acceptors. In redox-neutral reactions, the balance of electron-transfer steps between the reacting molecular partners allows construction of complex molecules in a more atom-economical fashion (*12*). In one of the redox-neutral approaches, both reactive partners are transformed into free-radical intermediates, and bond formation proceeds through radical-radical coupling.

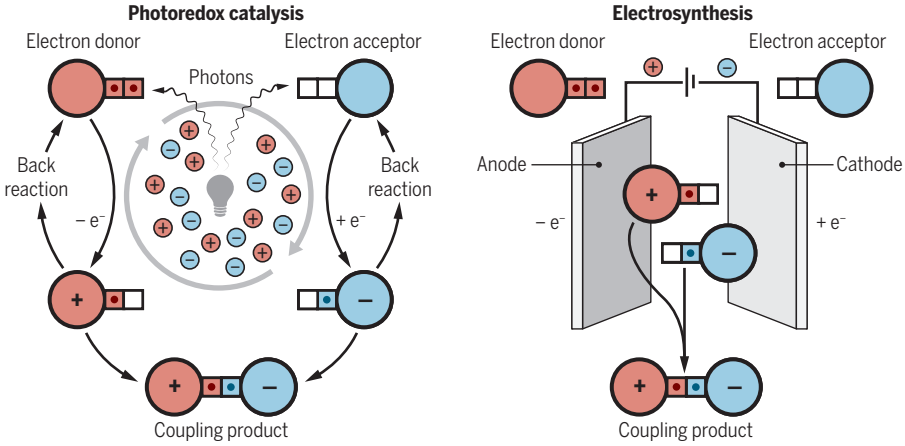
The activation barrier in such reactions predominantly stems from the oxidation and reduction steps and can be surmounted with the aid of photoredox catalysis or electrosynthesis. Either the energy of photons in photoredox setups or the potential energy between the electrodes in an electrosynthetic cell can drive the formation of radical intermediates. An important distinction between the two approaches is the spatial separation between the oxidation and reduction reaction sites (see the figure). In photoredox catalysis, the molecular photocatalyst acts as both the oxidizing and reducing agent and is freely diffusing in solution. In an electrosynthetic cell, the oxidation and reduction reactions are confined to the surface of the cell electrodes.

This distinction leads to profound differences in reactivity patterns and intrinsic limitations observed in both systems. For example, back-electron transfer can compete with the productive reaction pathways in photocatalytic systems, and the short half-life of certain free-radical species can restrict product formation in electrosynthetic cells. The latter challenge has been successfully addressed in the work by Mo *et al.* by introduction of a microfluidic redox-neutral electrochemistry (μRN-eChem) platform inspired by the previous developments in continuous-flow electrosynthesis (*13*, *14*). The disclosed approach relies on an extremely small interelectrode gap (down to 25 μm) in the electrosynthetic cell that substantially decreases the diffusion time between electrodes and intensifies the mass transport of unstable free-radical coupling partners generated on the electrodes. The decreased interelectrode gap also eliminates the need for a supporting electrolyte and redox mediators, commonly used to facilitate charge transfer between the electrodes and the species in solution.

These improvements allowed Mo *et al.* to use the μRN-eChem platform with reactions in which the free-radical intermediates are too unstable to allow their efficient coupling in conventional electrosynthetic cells or in photoredox systems. They executed redox-neutral coupling reactions with unstable carbon- and nitrogen-based radical intermediates, illustrating that the immense potential of radical-chemistry manifolds can be harnessed with an appropriate electrosynthetic cell design. To date, complex radical-based transformations were mainly demonstrated in photoredox settings, and far fewer synthetically challenging reactions were executed with electrosynthesis (*15*). The current work strives to eliminate this difference and provides synthetic chemists with a powerful tool for expanding the scope of redox-neutral free-radical reaction manifolds in electrochemical settings. ■

Approaches to redox-neutral radical-radical coupling

Photoredox and electrosynthetic approaches to radical-radical coupling reactions differ in fundamental ways. Both generate radicals through single-electron transfers that can undergo otherwise difficult coupling reactions.



Photocatalytic systems

The photogenerated oxidized and reduced radical species are distributed in solution and are subject to back reaction to the neutral species.

Electrosynthetic systems

The oxidation and reduction reactions are confined to the electrode surfaces. In the microfluidic system of Mo *et al.*, these species come together rapidly for reaction.

GRAPHIC: C. BICKEL/*SCIENCE*

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MEDICINE

Drug modulation by nuclear condensates

Concentration of antineoplastic agents into spatial compartments influences activity

By **Aaron D. Viny**[1,2] *and* **Ross L. Levine**[1,2]

Many mysteries remain about the eukaryotic nucleus. In the past decade, much has been discovered anew about the three-dimensional (3D) organization of the nucleus and the dynamic interactions therein that influence cellular function. Studies have uncovered the critical roles that topologic structure, histone modifications, and DNA modifications play in regulating transcription. By contrast, the understanding of interactions among proteins, RNA, and chromatin in macromolecular assemblies is less developed. These condensates are the molecular basis for discrete nuclear spatial organization of active and repressive chromatin as well as distinct nuclear structures such as the nucleolus (*1, 2*). On page 1386 of this issue, Klein *et al.* (*3*) begin to dissect the functional relevance of nuclear condensates in regulating the distribution and activity of fluorescent-labeled antineoplastic agents with specific nuclear localization profiles. This finding could have wide-ranging implications for the understanding of pharmacologic mechanisms and has substantive consequences for therapeutic development, drug delivery, and target engagement.

Recent technologies have allowed for appreciation of hierarchical 3D structures within the nucleus that have both known and unknown functional importance. Most widely accepted is the principle of spatial separation of chromatin into regions of active transcription (euchromatin). These regions share the property of deoxyribonuclease (DNase) or transposase accessibility and the presence of transcriptional coactivators, including the mediator (MED) complex. These genomic regions are spatially separate from regions of transcriptional inactivity (heterochromatin), densely packed histone-bound chromatin, often demarcated by repressive histone methylation marks.

Beyond euchromatin and heterochromatin, other segregated nuclear subcompartments have been described as self-organizing condensates. Through careful assessment of nuclear condensates (*4, 5*), Klein *et al.* observed that fluorescent-labeled small molecules had distinct and specific spatial aggregation in different nuclear compartments. Fluorescent-labeled cisplatin, a chemotherapy drug that damages and cross-links DNA, was observed to aggregate in mediator of RNA polymerase II transcription subunit 1 (MED1) transcriptional condensates while diffusing freely through heterochromatin protein 1 (HP1)–demarcated heterochromatin. Upon locus-specific assessment of cisplatin-induced DNA damage (platination) across the genome, only the DNA contained in MED1 condensates had high amounts of cisplatin incorporation. This finding suggests specificity of the pharmacologic effect of cisplatin to a distinct type of condensate. Additionally, the differential effect on platinum incorporation was lost with bromodomain-containing protein 4 (BRD4) inhibition, which disrupts MED1 condensates.

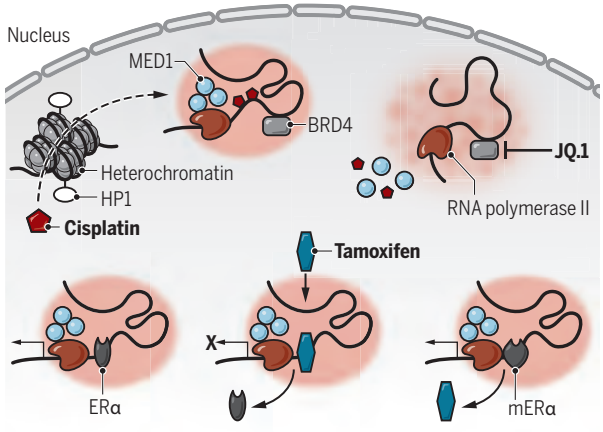
The authors also assessed the localization of tamoxifen and found that this targeted endocrine chemotherapy aggregated in MED1 condensates, competing with and evicting its target, estrogen receptor α (ERα), from these intranuclear structures. In human breast cancer cells, they found that ERα interacts with the *MYC* locus in MED1 condensates; this association was abrogated by tamoxifen and condensate accumulation. Notably, patient-derived ERα mutations that induce tamoxifen resistance led to their retention in MED1 condensates, rendering tamoxifen unable to outcompete oncogenic ERα.

Klein *et al.* demonstrate that a subset of small-molecule therapeutics can have differential enrichment in nuclear compartments (see the figure). Moreover, functional studies have demonstrated that redistribution of high-density regions of histone 3 lysine 27 acetylation (H3K27ac), consistent with transcription-activating super-enhancer function, within condensates may contribute to platinum resistance in human ovarian cancer cell lines (*6*). These two observations suggest an important, dynamic link between transcriptional units and epigenetic marks in nuclear condensates with pharmacologic agents possessing condensate-specific proclivities. These studies further suggest that the modulation of condensate structure and function has critical roles in gene regulation and therapeutic response. This raises the question of whether the localization and efficacy of cisplatin and of other small-molecule therapeutics are driven by transcriptional and epigenetic regulation, by the structure of nuclear condensates, or by these two dynamic processes acting in concert.

Undoubtedly, there remain technical limitations to the ability to assess and elucidate the relationship between nuclear structure and specific perturbations, particularly in dynamic contexts, including malignant transformation and the response to specific therapies. As a cell responds to intracellular and extracellular signals, transcription factor activity is influenced by both chromatin conformation and other dynamic factors that have a critical role in these same biologic processes. Notably, a recent description of genetic alterations in transcription factors was shown to affect

Pharmacophore-specific condensates

Cisplatin and tamoxifen aggregate in transcriptional condensates, affecting their pharmacokinetics. Cisplatin freely diffuses through HP1-labeled heterochromatin and accumulates in MED1 condensates. The BRD4 inhibitor JQ.1 abrogates MED1 condensates and attenuates drug-induced platination. Tamoxifen displaces ERα from target genes, but mutations that induce tamoxifen resistance (mERα) shift affinity as tamoxifen is evicted from transcriptional condensates.



BRD4, bromodomain-containing protein 4; ERα, estrogen receptor α; HP1, heterochromatin protein 1; MED1, mediator of RNA polymerase II transcription subunit 1.

GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

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spatial localization to transcriptional condensates (*7*). Indeed, transcription-directed compartmentalization may have a critical role in regulating condensates, an observation best illustrated through the effects of herpes simplex virus type I in altering nuclear organization through highly accessible viral DNA binding sites and sequestration of RNA polymerase II (*8*).

Consistent with this hypothesis, intrinsically disordered regions within transcription factors can contribute to altered nuclear localization in human disease, as has been shown in syndactyly (fused fingers) driven by an intrinsically disordered region repeat expansion in homeobox protein 13 (HOXD13) (*7*). As was shown by Klein *et al.* with respect to pharmacophore-specific condensates, fluorescence recovery after photobleaching (FRAP)–based techniques were used to elucidate altered condensate formation in HOXD13 expansion syndactyly. Although FRAP cannot fully distinguish liquid diffusion from high-affinity protein structures (*9*), the relative contribution of liquid-liquid phase separation versus protein structured "hubs" remains an exciting and controversial question with respect to nuclear condensate formation and function.

These recent studies have dissected the inner workings of the nucleus, which are as beautiful and fascinating as much as they are still beyond complete understanding with respect to regulatory function and purpose. Enormous potential lies in the ability to manipulate these characteristics for therapeutic intervention. It is anticipated that further research will soon reveal the mechanistic importance of nuclear phase separation in cellular function, and the molecular consequences of linking spatial compartmentalization with nuclear function. ■

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ECOLOGY

Using information theory to decode network coevolution

Communication clashes shape the coevolution of insect-plant ecosystems

By **Ricard Solé**

Walking through a forest, you spot a colorful butterfly larva crawling and munching on a leaf—nothing unusual, just one scene in a calm ecological play. And yet, a massive "arms race" rages between plants and their herbivores (*1*, *2*), spurred by information and misinformation transfer. Chemical signals play the role of communicators in a channel that joins each pair of interacting partners. The mechanisms that drive coevolution of these chemically mediated webs have been an active area of research, but a satisfactory theory has yet to be established. On page 1377 of this issue, Zu *et al.* (*3*) describe a new application of information theory to coevolutionary dynamics in animal-plant networks.

Many scholars have explored the role of information in ecology and evolution (*4*) since the 1950s, in the wake of Claude Shannon's groundbreaking theory of information and communication (*5*). But the actual function of information, particularly in an evolutionary context, often has been obscured by insufficient data and the lack of a proper mapping of the links between information and fitness (*6*).

Zu *et al.* made use of plants' secondary metabolism (which forms metabolites not involved in plant growth or development) to couple two bipartite networks, namely the animal-plant (AP) one (i.e., who eats what) and the plant–volatile organic compounds (PV) one [i.e., what volatile organic compounds (VOCs) plants generate]. The authors gathered data from a tropical dry forest; insect larvae were collected from leaves, and trophic interactions were confirmed in the laboratory. The VOC repertoire was retrieved from leaves in the field with a method that characterizes the chemical profile around each leaf.

However, the way the authors built a communication channel between the AP and PV networks departed from Shannon's approach in a substantial way. The classical theory considers a sender and a receiver trying to communicate using a given code sent through a noisy channel (Morse code in the old telegraph system would be one example). For each signal sent, there is a probability that it will be misunderstood by the receiver. Not surprisingly, a great deal of standard information theory deals with finding ways of optimizing communication efficiency. However, in an ecological system in which interactions are highly asymmetric (one species eats the other), the needs at the ends of the (coevolving) communication channel are in clear conflict: Insects need to faithfully identify what leaves are edible, and plants need to avoid being identified as edible.

The aim of Zu *et al.* was to connect information-level descriptions of the two networks through a simple coevolutionary model that successfully solves the problem of defining fitness values for both plants and insects. The rules of the model simulate genetic mutations that influence pairwise interactions within the two bipartite networks. In the simulation, a given PV pair was chosen at random. With a certain probability, the connection between the plant and the chemical signal was added (if there was no link) or removed (if there was one), thus increasing or decreasing the VOC repertoire, respectively. A fitness value, F_P, was then computed using one of Shannon's entropic measures, which weights the uncertainty associated with chemical recognition by animals. If the new fitness value, F'_P, is larger than the original (before the PV choice), the change is accepted, and the VOC repertoire is expanded.

This feature of Zu *et al.*'s coevolutionary model is particularly notable, as communication networks usually are intended to increase channel efficiency through noise reduction (maximizing information transfer between sender and receiver). In their model, however, changes in a plant's VOC repertoire expanded the chemical diversity of volatiles that favor the confusion of the herbivores or, in Shannon's theory, that increase the herbivores' uncertainty about what plant choices they can make.

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Information between an herbivore (such as a caterpillar) and plant is exchanged through volatile chemical signals, a communication that drives coevolutionary arms races.

PHOTO: ANTONIO LÓPEZ-CARRETERO

The same mutation process was then applied to the herbivore-plant matrix, but now the chosen pairs were AP (insect-plant) duos. These pairs were also added or removed using a fitness measure. In this case, the selection pressure takes place in the opposite direction, toward reduction of the range of alternative chemical signals. Fitness is thus defined from another entropic measure that weights the improvement of insect specialization obtained from the animal-VOC matrix, which is the product of information transfer between the PV and the AP networks. In this way, two optimization constraints—plant VOC production and insect specialization—must be satisfied simultaneously.

With their new model, Zu *et al.* calculated several information measures from sets of interactions between lists of pairwise exchanges. The authors also extracted probabilities of interactions as well as so-called conditional probabilities (e.g., the probability that a given insect will recognize a specific plant). The sets of probabilities were used to estimate the efficiency of coding and decoding strategies. These measures and the predicted network structure matched very well with those estimated from the field data, including the dense set of connections in the PV data as well as the pattern of insect specialization in the AP set. These results were independent of initial conditions, which included both sparse and dense interaction webs. Such agreement supports the proposal that coevolutionary dynamics is at play and illuminates how VOCs influence the fitness of both plants and herbivores (*7*).

Both matter (biomass) and energy (metabolism) are key to our understanding of life. Information stored in DNA and signals transmitted between individuals are major driving forces in biology (*8*). In ecology, information theory has delivered testable predictions in the study of energy-flow networks (*9*) and in characterizing the ecological specialization of plant-pollinator networks (*10*). The theoretical framework presented by Zu *et al.* explicitly connects coevolutionary dynamics and network architecture. This framework can be applied to microbial VOC–based communication associated with plants (*11*) or the evolutionary dynamics of the rhizosphere, where a diverse range of signaling processes connect plants, fungi, and microorganisms (*12*).

In a general context, this formal approach to network coevolution could aid efforts to formulate a theory of ecosystem learning through coevolution (*13*). Furthermore, the opposing pressures that shape the evolution of language networks—which is closely related to Zu *et al.*'s modeling efforts—also include simultaneous minimization of communication costs and generate statistical distributions that reveal universal laws in language organization (*14*). ■

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POLICY FORUM

EDUCATION

Understanding persistent gender gaps in STEM

Does achievement matter differently for men and women?

By **Joseph R. Cimpian[1], Taek H. Kim[1], Zachary T. McDermott[2]**

Gender gaps in science, technology, engineering, and math (STEM) college majors receive considerable attention, and it is increasingly recognized that not all STEM majors are equal in terms of gender disparities (*1*–*4*). For example, the male-to-female ratio among U.S. college majors in biology, chemistry, mathematics, and many other STEM fields is now about 1-to-1 (*2*, *5*), whereas in physics, engineering, and computer science (PECS), the ratio appears to have plateaued at about 4-to-1 (*2*, *4*, *5*). Here, we make two important contributions, showing (i) how gender relates to pursuit of a PECS degree throughout the achievement distribution and (ii) that student characteristics that predict PECS pursuit in the literature are not equally predictive of the gender gap throughout the achievement distribution. We find that a surprisingly large number of low-achieving men are majoring in PECS, relative to women, and this cannot be explained by an extensive set of student-level factors proposed in the prior literature (*6*–*10*). We can, however, explain the gender gap among high-achieving students. These patterns suggest that interventions to close the gender gap may work to attract high-achieving women; yet, something beyond these student factors may be attracting low-achieving men and repelling average- and low-achieving women, and without addressing those factors, it is unlikely that the PECS gender gap will fully close.

Despite historical emphasis on measuring whether gender gaps in STEM achievement exist and if there is greater male variability in achievement, there is surprisingly little research linking gender differences in achievement to pursuit of a college major [see supplementary materials (SM), section 1]. Work that links these factors accounts for average differences in STEM achievement and suggests that controlling for them does little to reduce the gender gaps in PECS, but it does not tell us where in the achievement distribution the PECS majors are coming from or if this differs for men and women.

We use new data from the U.S. Department of Education's High School Longitudinal Study of 2009 (HSLS:09), a nationally representative, longitudinal cohort of students who were in ninth grade during the 2009–2010 school year and were followed for 7 years. For several reasons, the HSLS:09 is the ideal dataset for examining who majors in PECS throughout the achievement distribution. First, it allows us to study a critical 7-year period, from the beginning of high school through the first few years of college when students declare majors (*3*, *11*). Second, its multiple measures of STEM achievement during high school allow for more comprehensive and robust measurement than most datasets. We combine these measures of STEM achievement into a composite STEM achievement variable with very high reliability (Cronbach's $\alpha = 0.86$; moreover, each individual measure yields a similar pattern of gender gaps; see fig. S1). Third, surveys conducted during the summer and fall of 2013 (after high school graduation for most) asked students about their college major intentions; then, they were asked 3 years later which major they were ultimately pursuing, allowing us to examine persistence in interest in a major and newfound interest in a major.

"...low-achieving men are both retained in and attracted to these fields during college..."

The final analytic dataset contains 5960 students, with complete data on majors and STEM achievement, and multiply-imputed data for any missing covariates. All analyses use attrition-adjusted longitudinal sampling weights to ensure national representativeness and to adjust standard errors to account for the multistage survey sampling design. Detailed methods are in the SM. Although the data do not capture degree completion, they are likely a reasonable proxy, because research suggests that persistence to STEM degree is similar for both genders (see SM, section 2). The data reflect the United States, not international contexts.

GAPS DIFFER AT THE TOP VERSUS BOTTOM

Of all U.S. students attending a 4-year college, 23.8% of men and 5.5% of women pursued a PECS major, representing a substantial and significant gender gap on average ($p < 0.001$). When ranked from lowest to highest achieving, we see that males at and below the 1st percentile are majoring in PECS at the same rate as females at the 80th percentile of STEM achievement (see the figure, top). At every point in the STEM achievement distribution, men majored in PECS at higher rates than did women (all values of $p < 0.004$). The raw percentage-point gap in the top third of the achievement distribution is about twice the size of the gap in the bottom third (23 versus 12%, all values of $p < 0.001$; see Model 1 in the table). The size of these gaps is worth noting when thinking about the scale of the disparity throughout the distribution; however, percentage-point gaps do not factor in base rates, and thus, the gap represented in ratios is critical when examining representation. Importantly, the ratio at the bottom of the distribution is much larger than at the top. Overall, the male-to-female ratio in PECS is 4-to-1, whereas at the bottom decile, it is greater than 10-to-1 and at the top decile less than 2-to-1 (see the figure, top). This point is critical for considering retention in the PECS "pipeline" and should fundamentally shift how progress toward gender equity is evaluated.

Who majors in PECS can be disaggregated further by intention just after high school graduation to major in PECS: students who intended to major in PECS versus those who had different intentions (see the figure, middle and bottom). More males intended to major in PECS than did females (23.7 versus 5.2%, $p < 0.001$). Although persistence rates from post–high school intention to college major are similar overall (74 versus 75% for men and women, respectively; $p = 0.87$), these rates mask a substantial gender differential throughout the distribution, where females and males persisted at similar rates among the highest achievers but males persisted more among the lowest

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achievers (see the figure, middle). Indeed, among PECS-intenders who persisted, females averaged almost nine percentiles higher on the STEM composite measure (p = 0.004; table S1), because the male average was brought down by low achievers who persisted. This pattern of men's persistence being less sensitive to feedback on their actual performance is consistent with a general pattern found in other recent work (see SM, section 3). Importantly, males not previously intending to major in PECS join these majors at significantly higher rates than females at all points in the distribution (all values of $p < 0.008$; see the figure, bottom). Among non-PECS-intenders, males scoring at and below the 1st percentile were at least as likely to join PECS as females scoring above the 99th percentile who did not initially intend to major in PECS.

Supplemental analyses disaggregated PECS into physics only, engineering only, and computer science only (figs. S2 to S4), and common (although not identical) patterns were found, supporting the combining of majors for this main analysis.

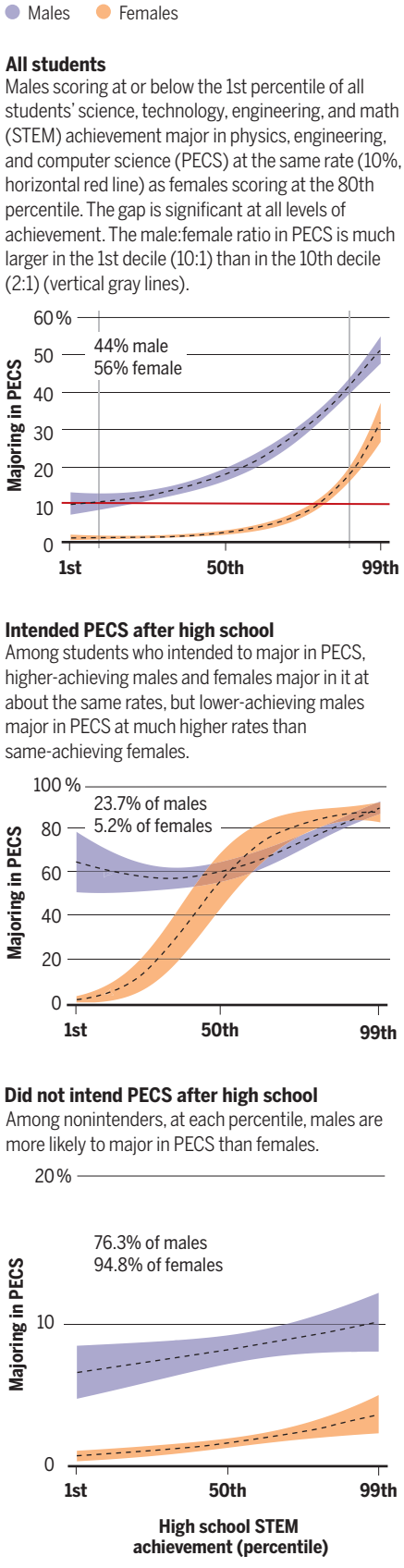
EXPLANATION GAP AT THE BOTTOM

Are these gaps explained by other differences between men and women? Prior research suggests that women may choose PECS (and STEM, more generally) less often than men because they have lower confidence in their math abilities (*6*), have other options owing to a comparative advantage in English and/or reading (*7*), value societal goals and a work-life balance more than salary (*8*), take different high school STEM courses (*9*), and have different career aspirations (*10*).

Our ability to statistically account for the PECS gender gap with these and other variables depends on whether we look at low-, average-, or high-achieving students (see the table and fig. S5). Overall, our model that simultaneously adjusts for a range of student attributes explains almost all of the gender gap among high achievers, but not a substantial amount among low achievers. Among the top third of achievers, the average male-female gap goes from 23.0% (p < 0.001) in the unadjusted model to just 0.3% and no longer significant (p = 0.84) in the final adjusted model, for a total reduction of 98.6% [i.e., equal to (23.0% – 0.3%)/23.0%]. The rich set of covariates, however, does not do as well predicting the gaps among average- or low-achieving students. For example, the gap among the bottom third reduced only from 11.7 to 7.7% and remained significant (p < 0.001) after all adjustments (see the table). All patterns are robust to a wide range of sensitivity checks, including mean replacement for missing data, not using sampling weights, using cross-sectional weights, nonparametric models, and restricting the sample to students in colleges with engineering programs (figs. S6 and S7).

Who majors in PECS?

The shaded areas represent one standard error above and below the estimated means.


Males
Females
All students
Males scoring at or below the 1st percentile of all students' science, technology, engineering, and math (STEM) achievement major in physics, engineering, and computer science (PECS) at the same rate (10%, horizontal red line) as females scoring at the 80th percentile. The gap is significant at all levels of achievement. The male:female ratio in PECS is much larger in the 1st decile (10:1) than in the 10th decile (2:1) (vertical gray lines).
60 %
50
40
30
20
10
0
Majoring in PECS
44% male
56% female
1st
50th
99th
Intended PECS after high school
Among students who intended to major in PECS, higher-achieving males and females major in it at about the same rates, but lower-achieving males major in PECS at much higher rates than same-achieving females.
100 %
80
60
40
20
0
Majoring in PECS
23.7% of males
5.2% of females
1st
50th
99th
Did not intend PECS after high school
Among nonintenders, at each percentile, males are more likely to major in PECS than females.
20 %
10
0
Majoring in PECS
76.3% of males
94.8% of females
1st
50th
99th
High school STEM achievement (percentile)

NON-PECS AND RACE

We examine gender gaps in other STEM majors and race gaps in STEM as a point of comparison for our PECS-focused analyses (fig. S5 and tables S2 and S6 to S9. We find no significant gender gaps on average or throughout the achievement distribution in other STEM majors (table S11) after adjusting for all covariates. Similarly, there are no significant race gaps [Asian or white versus underrepresented racial minorities (URM)] in either PECS or other STEM, either on average or throughout the distribution after an extensive set of controls. It is also worth exploring gender-by-race patterns in PECS (*12*). The data revealed notable consistencies in gender gaps when looking just among Asian or white individuals or just among URM race groupings (figs. S8 and S9).

IMPLICATIONS

This new work reveals the critical importance of assessing the gender balance in PECS throughout the achievement distribution, rather than averages. Here, we discuss how this distributional approach (i) can transform how the field evaluates gender equity in the STEM pipeline and (ii) differentially affects how to target interventions to high versus average and low achievers. Ultimately, this approach suggests that student-level factors are complex and can vary across achievement levels. We urge the field to continue to look beyond student-level factors—toward discipline and societal ones—to further reduce the gender gap and raise the overall achievement of students in PECS.

IMPLICATIONS FOR THE STEM PIPELINE

Some have argued that the STEM pipeline from undergraduate to graduate school has stopped "leaking" because the proportion of females in STEM at the undergraduate level has remained constant to the graduate level (*1*, *13*). Our analyses suggest that looking at average persistence rates may obscure differential leaking throughout the achievement distribution. If PECS graduate programs selected from the highest-achieving students solely on the basis of merit, we should expect to see a considerably higher proportion of females in PECS graduate programs than in undergraduate programs. For example, if graduate programs select students on the basis of STEM achievement alone from the top decile, where the male-to-female ratio is less than 2-to-1, we should

GRAPHIC: X. LIU/*SCIENCE*

expect to see more than 33% females in graduate school, not 20%, which is the average during undergraduate school.

Thus, the apparent lack of a leaking pipeline on average found in previous studies (*1*, *13*) is not the appropriate standard for evaluating equity because it ignores that female PECS majors were initially higher achieving. Furthermore, evidence of differential leaking throughout the distribution could help reconcile seemingly disparate findings, such as a pipeline that no longer leaks on average (*1*, *13*) and male-dominated fields holding beliefs that deter females while sending welcoming signals to lower-achieving males for graduate education (*2*, *14*). It may be precisely through a male-favoring culture that graduate PECS programs keep the proportion of females at the same low rates as they are during undergraduate school rather than allowing them to rise, as would be expected given females' aforementioned higher competence.

Modeling the gender gap in PECS

		BY TERTILES OF HIGH SCHOOL STEM ACHIEVEMENT		
	OVERALL	LOW	AVERAGE	HIGH
MODEL 1: Unadjusted gap	18.2***	11.7***	17.2***	23.0***
MODEL 2: STEM composite adjustments	16.1***	10.3***	15.6***	22.3***
MODEL 3: Student attributes and attitudes	9.9***	9.5***	11.2***	9.0***
MODEL 4: Aspirations and intentions	5.5***	7.4***	5.8***	3.2*
MODEL 5: All adjustments	4.1***	7.7***	4.5**	0.3

All gaps are expressed in percentages. All estimates are average marginal effects, following logistic regressions predicting the major as a function of gender (Model 1), throughout the achievement distribution (Model 2, and subsequent models). Model 3 adjusts for factors of math and science interest, identity, self-efficacy, and utility, reported in grades 9 and 11, all interacted with achievement. Model 4 adjusts for college major intentions and occupational aspirations at age 30, reported in both grades 9 and 11. Model 5 adjusts for all variables listed above. See supplementary materials for details and for the underlying logistic regression output.
***$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.**

IMPLICATIONS FOR ACHIEVEMENT LEVELS

The differential ability of covariates to explain the PECS gender gap at different points in the achievement distribution has direct implications for how researchers and educators should intervene.

For high-achieving students, our analyses suggest that efforts to boost women's PECS aspirations and intentions (i.e., the strongest set of predictors)—such as role-model interventions and Girls Who Code (*4*, *15*)—may work well to eliminate the gender gap in pursuing PECS at the top of the distribution. That is, although each set of variables chipped away at the gender gap at the top of the distribution, none of them—alone or collectively—were as strong or consistent a predictor as prior intention to major in PECS and having aspirations for a PECS-related career (see the table, fig. S5A, and table S6). This clearly implies that to close the PECS-pursuit gender gap among the top achievers, the critical piece is in converting all other STEM-positive factors (e.g., confidence and interest) into actual aspirations and intentions.

Unfortunately, for average- and low-achieving students, our analyses also revealed that the field's understanding of how to get average- and low-achieving girls into the field is more limited. The same factors that explained the PECS gender gap at the top of the distribution collectively explained less and less of the gap further down the distribution. Thus, the interventions that may work for high-achieving girls are unlikely to work for lower-achieving ones. Moreover, focusing efforts on attracting high-achieving women to PECS may backfire: Whereas men have male peers to serve as role models throughout the achievement distribution, having only high-achieving role models for women may send signals to average- and low-achieving women that they do not have the necessary STEM skills to succeed (see SM, section 4).

Disaggregating PECS majors by achievement tiers raises a related question: Should PECS be attracting and retaining low-STEM-achieving students (of any gender), or is there an alternative pathway to meet the PECS labor market demands with more competent students? Students in the lowest third of achievement have mean math SAT scores and high-school STEM grade point averages that are well below average, especially for these math-intensive fields. About 90% of the students majoring in PECS from this tier are men, and we have shown that low-achieving men are both retained in and attracted to these fields during college (see the figure, middle and bottom, and figs. S3 and S4). Thus, the question about whether PECS wants low-achieving students is inextricably intertwined with a different question: Why are men—and not women—with low achievement more drawn to these fields? Although we have shown that this question cannot be answered by gender differences in a wide range of student-level factors—including self-efficacy, math or science identity, and earlier aspirations—it is possible that the masculine culture of these fields and the gender stereotypes attached to PECS (*2*, *4*, *11*, *14*, *15*) may lead to retaining less-qualified men over more-qualified women. Thus, it is important for PECS fields to break down the barriers for women to enter the field and, in doing so, raise the overall quality of the students entering these fields.

All of this suggests that, although role-model interventions, coding camps, and fostering strong peer networks are worth pursuing, there are real obstacles to achieving gender parity in PECS that transcend these interventions, which may work only for a select group of high-achieving women. To develop effective interventions for gender equity and raise the overall quality of students in PECS, it is critical to recognize that the gender imbalance varies throughout the achievement distribution and may be perpetuated by male-favoring cultures that disproportionately attract low-achieving men to the field. ■

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6497/1317/suppl/DC1

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BOOKS *et al.*

Biologist Nancy Hopkins used a data-driven approach to document subtle sexism at MIT in the early 1990s.

SCIENCE LIVES

Creating a culture of change

Urging action, a new film paints a harrowing portrait of female scientists' experiences in academia

By **Donna Riley**

Sweeping in scope yet intimately compelling, *Picture a Scientist* tells the stories of three female scholars, revealing the systemic and structural nature of gender discrimination and harassment in academic science. The film shows how intersections of sexism and racism shape experiences differently for white women and for women of color and how implicit bias both generates inequity and prevents us from noticing it. It reveals injustices ranging from subtle slights and salary gaps to bullying and physical assault. And it reminds us of the power of women's collective action; the value of social science research in analyzing and responding to inequality; and the importance of allies and advocates, especially in university leadership.

Picture a Scientist
Ian Cheney and Sharon Shattuck, directors
Uprising LLC, 95 minutes.
Streaming via select theaters 12–26 June 2020.

Biologist Nancy Hopkins shows what convivial conspiracies can hatch over lunch with a colleague, when one dares to act. In the early 1990s, convinced that male colleagues at the Massachusetts Institute of Technology (MIT) were afforded more lab space than their female counterparts, she took a tape measure to her own lab and to the lab spaces of male colleagues in the dead of night, carefully documenting the disparity. With this data-driven approach, Hopkins gained the support of her female colleagues and upper-level administrators and went on to lead a more comprehensive study investigating salary gaps, access to day care, and other inequities in need of redress (*1*).

But the sexism Hopkins experienced during her career was not always subtle. In the film, she recounts a shocking groping incident that occurred when she was an undergraduate presenting her work to a visiting colleague. Stunned by the encounter and certain that no one would believe her over her perpetrator, she resolved to act as though nothing unusual had occurred, answering the Nobelist's questions without mention of the offense.

Sexual harassment is more often a "put-down" than a "come-on," explains anthropologist Kate Clancy in the film's narration, as geoscientist Jane Willenbring reveals how she was bullied, humiliated, and assaulted while conducting fieldwork in Antarctica as a graduate student in 1999. After nearly two decades of silence, she felt compelled to speak out against the perpetrator when her young daughter began expressing scientific aspirations of her own. Willenbring's Title IX complaint resulted in her perpetrator's dismissal, due in part to the supporting testimony of a bystander, graduate student Adam Lewis. One wonders why Lewis did not intervene at the time, but as he shares his epiphanies and takes gentle correction from his colleague, we see how true allies are formed.

What a ponderous thing it is to hold an esteemed scientist accountable for unprofessional behavior; so much has to align to achieve any semblance of justice. For every story, there are thousands left untold; academia is haunted by those who cannot come forward, those who are not believed, and those who are dismissed as ill-suited for the discipline.

In chemist Raychelle Burks's story, we encounter the toll of day-to-day slights, underestimations, and the simultaneous invisibility and hypervisibility that women of color experience in the academy. As Burks recounts how a colleague challenged her right to park in the faculty lot, we see how such experiences can accrue over one's career, taking time and energy away from science. Psychologists Mahzarin Banaji and Corinne Moss-Racusin reveal how our implicit biases prevent us from associating women with excellence in science, generating inequity that slips by unnoticed unless we are vigilant.

Picture a Scientist is a world-class documentary from an experienced, award-winning crew that tells harrowing truths without sugarcoating, sensationalizing, or objectifying the film's subjects. If I must have a quibble, it is that the film's treatment of race is limited to black and white and its treatment of gender is too binary. Other axes of difference, such as disability, class, or sexual orientation, go largely unaddressed. Still, the film leaves openings to discuss these omissions and, more importantly, compels us to action. We might ask ourselves: What data could we gather on our own campuses? How do we become the accomplices of change-seeking colleagues? Can we muster the courage to share our own stories or hold someone accountable rather than looking the other way?

Picture a Scientist will be available to stream via select U.S. theaters from 12 to 26 June 2020. Invite campus leaders to attend the virtual premiere, and then host a screening and organizing session (*2*). This film could be the vehicle that moves us, as Clancy advocates, "away from a culture of compliance and towards a culture of change." ■

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PHOTO: UPRISING LLC

PSYCHOLOGY

What to do with our days

When wielded correctly, boredom can be a powerful tool

By **Erin Westgate**

On 13 May 1982, Valentin Lebedev was launched into space, where he would spend a record-breaking 211 days. It took only a week for the astronaut to confront a surprising problem: He was bored. Lamenting the "drab routine" and tedious "busy work," his space diary documents his struggles and his intense anticipation of a video camera that he hoped would relieve his boredom.

Although we may not be adrift in space, boredom is often inescapable these days, as social distancing guidelines keep many of us at home. The key to mastering boredom, write John Eastwood and James Danckert in their intriguing new book *Out of My Skull*, is understanding its purpose. They argue that boredom is an important signal that "we need to act to fully realize our potential."

Eastwood, a clinical psychologist at York University, and Danckert, a cognitive neuroscientist at the University of Waterloo, introduce boredom as "the uncomfortable feeling of wanting, but being unable, to engage in satisfying activity." Defining it as a "feeling of thinking," they argue that boredom stems from four situational sources: monotony, constraint, lack of purpose, and lack of optimal challenge. When these contexts interact with individual internal states, such as motivation and attentional capacity, the same conditions can be experienced as either boring or deeply engaging.

Drawing a rigorous distinction between the feeling of being bored ("boredom") and the tendency for some people to be bored more easily than others ("boredom proneness"), Eastwood and Danckert systematically review empirical evidence in an attempt to determine whether boredom is good or bad, ultimately concluding that it is neither. Just like play, they argue, boredom serves important functions across species, guiding behavior away from inaction, toward novel and potentially rewarding outcomes.

Bored minks, for instance, behave very much like bored humans in lab experiments. When housed in unenriched cages, they become curious about exploring any and all new objects, from plastic water bottles to a moving toothbrush (which, the authors assure us, a mink finds quite exciting). And, like humans who are willing to self-administer painful electric shocks when bored (*1*), minks, too, show as much interest in aversive items, such as the smell of a predator, as they do in more positive ones.

Evidence that boredom propels exploration and learning also emerges from computer science. Programming artificial intelligence with the capacity for boredom provides a self-regulatory feedback loop that encourages optimal challenge and discourages programs from persisting at tasks that are either too complex to be feasible or too easy to offer new opportunities for insight. Indeed, when pitted against each other in computational models, it is boredom, not curiosity, that drives learning agents to make the most discoveries.

What does this mean in an age when we can easily suppress boredom with a few swipes on a smartphone? Eastwood and Danckert urge readers to heed the possibility that technology may offer a tempting but temporary solution. Brushing boredom away with "solutions," such as social media, does nothing to solve the lack of purposeful engagement that gives rise to boredom in the first place. While skeptical of claims that boredom is particularly harmful or beneficial—duly noting that experimental evidence is still in its infancy—they argue that we should take it seriously, by making room for it in our lives and responding appropriately when it arises.

One clue to remedying boredom lies in its emotional opposites: interest, enjoyment, and optimal "flow" experiences. By occupying our minds with activities we care about and finding ways to effectively pursue our desires and goals, we can act not only to address boredom as it occurs but also to prevent its occurrence in the first place.

Although Eastwood and Danckert take pains to clarify that they do not consider boredom to be an emotion, their approach is highly consistent with modern theories in social psychology and affective science that define emotions as "situated affective reactions," whose purpose is to provide information about whatever is in mind at the moment, our own thinking included (*2*–*5*). Drawing from this broader literature on emotion, and related work on the psychology of meaning and motivation, would have enriched the book's already broad approach to understanding what boredom is, why we experience it, and what happens when we do. Overall, however, Eastwood and Danckert's synthesis is an engaging and timely read that is anything but boring. ■

The same conditions can be experienced as either boring or deeply engaging, depending on our internal state.

PHOTO: ISTOCK.COM/PATRICKHEAGNEY

Out of My Skull: The Psychology of Boredom
James Danckert and John D. Eastwood
Harvard University Press, 2020. 288 pp.

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LETTERS

Edited by **Jennifer Sills**

Academic societies' role in curbing police brutality

The shocking death of George Floyd is unfortunately not a singular event in the United States. Eric Garner, Michael Brown, Freddie Gray, Amadou Diallo (*1*), and Breonna Taylor (*2*) are just a few examples from a long list of unarmed Black people who have died at the hands of the police. This pattern of violence is deeply rooted in the history of Black-White relations in the United States and the failure of the leaders of this country to deal with systemic racism. As professional physicists, we suggest that professional science and humanities organizations such as the American Physical Society (APS) take action by refusing to hold conferences in cities where police brutality takes place.

The APS and many other professional societies host large meetings in cities across the United States. APS cancelled a 2018 meeting in Charlotte, North Carolina, to oppose a law that discriminated against the LGBT community (*3*). The American Association for the Advancement of Science (the publisher of *Science*) has also selected cities for meetings based on their record of civil rights, including excluding the "Jim Crow" South from consideration and supporting states that ratified the Equal Rights Amendment (*4*). In this spirit, professional societies should protect Black people by meeting only in cities that implement evidence-based policies to limit the use of force by police. Academic societies should also assess cities' responses to police brutality, such as the transparency of communication and whether offending officers have been fired and charged with a crime. Social scientists have researched policies that can reduce police violence [e.g., (*5*)], and we encourage science societies to use this evidence to develop a set of criteria.

Economic pressure has historically been an effective strategy in bringing about social change. A decision to stop doing business in cities with inadequate protective policies would be similar to the divestment movement in South Africa in the 1980s (*6*), which was pivotal in ending apartheid. It would also be in keeping with Dr. Martin Luther King Jr.'s boycott of businesses in the South that refused to hire Black people (*7*). Academic societies should encourage positive change by sending a powerful message: Cities that reduce police brutality through egalitarian reforms will yield substantial financial benefits.

By using their power of the purse to oppose racist policing, professional societies would also be protecting their minority members, who might, for example, decide during a meeting to take a run through city streets while being Black. The ongoing protests demonstrate that established norms of social and race relations are changing. It is time for professional societies to live up to their charge of positively affecting society by taking a stand against police brutality in deed as well as in word.

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Pandemics' historical role in creating inequality

In her News Feature "An unequal blow" (15 May, p. 700), L. Wade explores how past pandemics disproportionately affected marginalized groups. It is important to recognize that in addition to exacerbating marginalization, pandemics set the stage for inequalities that have persisted for centuries (*1*). Historically, pathogens introduced by European conquistadors and colonists contributed to Indigenous population collapses throughout the Americas, facilitating colonial exploitation (*2*). Abrupt declines in the health and size of Indigenous populations, socio-cultural dislocation, and opportunistic grabs for resources became a strategy used by successive colonial governments (*3*). As scientists, we have a role in dismantling this colonial playbook to prevent coronavirus disease 2019 (COVID-19) from further marginalizing Indigenous communities.

Today's Indigenous peoples are facing a pandemic crisis. In North America, the Navajo Nation battles the virus across a vast region with irregular access to health services as a result of insufficient federal funding (*4*, *5*). The United States relies on agricultural laborers from Central America to maintain its supply chains but is deporting contagious workers (*6*, *7*), thereby seeding outbreaks in Mexico, where the disenfranchisement of 22 million Indigenous people makes the spread of disease difficult to control (*8*). In the Brazilian Amazon, Manaus is a national COVID-19 hotspot. The health system is overwhelmed, and Indigenous people previously displaced by the local government struggle to avert a 21st-century demographic collapse while fighting seizure of their lands (*9*).

Solving the crisis demands more than overhauling health infrastructure; it requires changing power relations to redress structural inequalities borne of colonialism. The path forward involves listening to Indigenous communities, promoting human-centered investment in sustainable development, and enforcing treaties and Indigenous land rights. For scientists, COVID-19 does not justify perpetuating extractive practices or pressuring communities to engage in research (*10*). Acknowledging Indigenous sovereignty is now more vital than ever. We must seize the opportunity this pandemic presents for societal transformation: Only through Indigenous self-determination, collaboration, and research partnerships rooted in

PHOTO: MICHAEL DANTAS/AFP/GETTY IMAGES

COVID-19 victims are buried daily in this cemetery in Manaus, Brazil, where the health system has been overwhelmed.

mutual respect can the Americas write a new playbook that fulfills the often stated but seldom realized ideals of equality for all.

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Recent immigrants at increased pandemic risk

In her News Feature "An unequal blow" (15 May, p. 700), L. Wade discusses the vulnerability of poor people and members of minorities during both past and present pandemics. However, the article overlooks recent immigrants, who have also been particularly vulnerable to pandemics in the past and are threatened now by the coronavirus disease 2019 (COVID-19) pandemic.

Human migration was also taking place during epidemics of the past. During the Black Death, some of those who died in London originated from distant portions of Britain (*1*), and rural refugees in Cairo, Egypt, exhibited a higher susceptibility to death (*2*). During plagues of the late preindustrial era in Milan, Italy, the death rate was higher among poor people originating from surrounding villages (*3*). In Dijon, France, during early recurrences of the Black Death, poor newcomers were more likely to die than were poor people who were long established in the city or who had recently settled but were members of established families (*4*).

The COVID-19 pandemic poses similar risks. In the U.S. states with newer Latino communities, Latinos have tested positive at higher rates than people with comparable income. In contrast, in some states with long-established Latino communities, Latinos are getting sick at rates similar to other population groups (*5*).

Although the comparative importance of frailty factors accounting for the present situation is a matter of debate, socio-economic and environmental factors are essential (*6*). This is consistent with the long-term historical perspective: During preindustrial European plagues, the vulnerable short-range migrants likely differed from the host population only in housing and working conditions. As past and present data demonstrate, recent immigrants should be included in discussions of risks to marginalized groups.

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RESEARCH

IN *SCIENCE* JOURNALS

Edited by **Michael Funk**

GEOPHYSICS

Seismic swarms show the structure

Faults responsible for earthquakes are idealized into two dimensions, despite fault zones being complicated, three-dimensional structures. Ross *et al.* used machine learning to find 22,000 seismic events near Cahuilla, California, during a seismic swarm. They used the locations and sizes of these events to show how the complex structure of the fault interacted with natural fluid injections from below. The authors' methods highlight the complexities of one fault and suggest a way to characterize other faults around the world. —BG *Science*, this issue p. 1357

A line marks the site of an ancient lake in a fault-rich region near Salton City, California, not far from the site of a recent seismic swarm induced by fluid injection from a deep reservoir.

CORONAVIRUS

Promising antiviral protease inhibitors

With no vaccine or proven effective drug against the virus that causes coronavirus disease 2019 (COVID-19), scientists are racing to find clinical antiviral treatments. A promising drug target is the viral main protease M^{pro}, which plays a key role in viral replication and transcription. Dai *et al*. designed two inhibitors, 11a and 11b, based on analyzing the structure of the M^{pro} active site. Both strongly inhibited the activity of M^{pro} and showed good antiviral activity in cell culture. Compound 11a had better pharmacokinetic properties and low toxicity when tested in mice and dogs, suggesting that this compound is a promising drug candidate. —VV

Science, this issue p. 1331

METALLOENZYMES

Delicate dance becomes a ballet

The enzyme nitrogenase uses adenosine triphosphate and several unusual iron-sulfur cofactors to pump electrons into typically inert dinitrogen (N_2), providing protons along the way. Previous work has shown that sulfur atoms in the iron-molybdenum cofactor (FeMoCo) are labile and suggests that replacement of one of the sulfurs by N_2 is integral to the mechanism of N_2 binding and reduction. Through the elimination of excess reducing agent during preparation, Kang *et al*. determined structures of Mo-nitrogenase in a resting conformation. Unexpectedly, they found that all three sulfurs at the outer edge of FeMoCo appear to be labile, with one subunit even having two of three sulfurs replaced by light, diatomic ligands. Biochemical and spectroscopic data indicate that the protein is active, holds tightly bound N_2, and is in the expected oxidation state. These results may prompt a reassessment of the possible mechanisms of N_2 reduction and the role of dynamic belt ligands in FeMoCo. —MAF

Science, this issue p. 1381

METALLURGY

Strong and tough steel

Ultrahard materials often do not have similarly impressive fracture toughness. Liu *et al*. discovered a processing route for medium manganese steel alloy in which ultrahigh strength accompanies high fracture toughness. The steel relies on both transformation-induced plasticity and delamination toughening to boost the fracture properties. The steel is composed of less expensive elements, making it

PHOTO: DAVID MCNEW/GETTY IMAGES

a potentially inexpensive material attractive for structural applications. —BG

Science, this issue p. 1347

CANCER THERAPY

Speedy screen for tumor therapies

Cell death screens using patient biopsies could be used to identify effective treatments for cancerous solid tumors. However, it takes several days to obtain results, meaning that the cells guiding treatment decisions may become molecularly distinct from those in patients. Bhola *et al*. developed a high-throughput method that, within 24 hours, identifies drugs that initiate cell death programs in tumor cells from freshly isolated patient biopsies. This method identified drug therapies that shrank breast and colon tumors in mice, but which would not have seemed as promising when screened in cells cultured for several days. —LKF

Sci. Signal. **13**, eaay1451 (2020).

INFLUENZA

Resistance to influenza antibodies

Broadly neutralizing human antibodies (bnAbs) to the stem of hemagglutinin (HA), a trimeric glycoprotein found on the surface of influenza viruses, are valuable therapeutics and can guide the development of universal influenza vaccines. For their use in therapy development, it is important to understand the extent to which HA stem variants with resistance to bnAbs can develop. Wu *et al*. used saturation mutagenesis combined with next-generation sequencing to systematically search for resistance mutations to prototypic bnAbs in two influenza subtypes, H3 and H1. They found that the genetic barrier to resistance to stem bnAbs was low for the H3 subtype but higher for the H1 subtype. The ability of H3 to develop resistance to bnAbs presents a challenge in the development of a universal influenza vaccine. —VV

Science, this issue p. 1335

BIODIVERSITY CHANGE

Land-use change and forest biodiversity

Land-use change by humans, particularly forest loss, is influencing Earth's biodiversity through time. To assess the influence of forest loss on population and biodiversity change, Daskalova *et al*. integrated data from more than 6000 time series of species' abundance, richness, and composition in ecological assemblages around the world. Forest loss leads to both positive and negative responses of populations and biodiversity, and the temporal lags in population and biodiversity change after forest loss can extend up to half a century. Land-use change precipitates divergent population and biodiversity change. This analysis has consequences for projections of human impact, ongoing conservation, and assessments of biodiversity change. —AMS

Science, this issue p. 1341

ELECTROCHEMISTRY

Cutting it close for radical coupling

In principle, electrochemistry is an ideal method for radical coupling: One precursor oxidized at the anode pairs up with a counterpart that has been reduced at the cathode. The trouble is that either or both coupling partners might not stay stable long enough to meet in the middle. Mo *et al*. resolved this issue by closely spacing the electrodes in a microfluidics platform (see the Perspective by Liu *et al*.). They showcase coupling of dicyanobenzene as the cathodic radical precursor with a variety of oxidatively generated partners. —JSY

Science, this issue p. 1352; see also p. 1312

IN OTHER JOURNALS

Edited by **Caroline Ash** *and* **Jesse Smith**

Scanning transmission electron micrograph of wire-shaped morphology in BiSeI.

THERMAL CONDUCTIVITY

Simple structures to slow heating

Materials with low thermal conductivity are important as barrier coatings and for other applications. Wang *et al.* found a bismuth selenohalide, BiSeI, with an extremely low thermal conductivity over a range of temperatures. The authors attributed their observations to the simple crystal structure of the material, along with other features that inhibited thermal transport. The characteristics that give bismuth selenohalide a low thermal conductivity could constitute a template for finding other materials that are good thermal barrier coatings or those that could be used for thermoelectrics. —BG

Sci. China Mater. 10.1007/s40843-020-1407-x (2020).

CORAL REEFS

Rot in the national bedrock

Coral reefs face numerous threats in today's changing environment. For low-lying atoll countries built on reef systems, the consequences can be catastrophic. The best-recognized threat to coral is climate change, but not all damage is outside local control. Local pollution seems to be taking a considerable toll in Tuvalu, a country in the South Pacific composed of several coral reef atolls. Nakamura *et al.* examined the composition of Tuvalu coral cores spanning decades. Since the 1990s, annual growth layers have been punctuated by black bands containing heavy metals, notably redox iron, and the genetic remnants of anaerobic bacteria. The authors

IMAGE: WANG *ET AL.*

FORESTRY

Genetic variation in boreal conifers

Boreal forests are suffering from the effects of climate change. Trees take decades to grow, and it is difficult to test their adaptive capacity. Depardieu *et al.* took advantage of a common garden experiment set up in 1979 in which tree seeds from 43 geographic origins were planted in a single location in Quebec, Canada. Tree-ring data from white spruce cores in 2006 showed growth responses to droughts in 1997, 2001–2002, and 2005. Trees from locations with drier climates displayed alterations in xylem anatomy and better recovery after drought than trees originating from more humid locations. These variations may reflect genetic adaption to local climate conditions in this widely distributed northern conifer. This information will be valuable in efforts to offset drought sensitivity in reafforestation planning. —PJH

New Phytol. **227**, 427 (2020).

White spruce trees (*Picea glauca*) native to the boreal forests of North America show genetic adaptation to the local environment.

infer that the black bands are produced by annually occurring anoxic conditions caused by pollution-driven algal overgrowth and coral suffocation. Such direct evidence of local conditions eroding the very bedrock of a country may provide enough motivation for change. —SNV

Sci. Rep. **10**, 7338 (2020).

CELL BIOLOGY

Monitoring mitophagy

Mitophagy is the autophagic process by which mitochondria are degraded in lysosomes. It removes dysfunctional mitochondria that accumulate in a variety of human diseases. New tools are needed to quantify autophagy and mitophagy in living cells. Katayama *et al.* have developed a signal-retaining fluorescent autophagy indicator (SRAI). The signal is not affected by delivery to the low-pH lysosomal environment and can be used in fixed as well as live samples. In a mouse model of Parkinson's disease, the indicator revealed that dopaminergic neurons selectively failed to execute mitophagy. This method will be useful in providing a reliable readout of mitophagy in a range of experimental biomedical settings. —SMH

Cell **181**, 1176 (2020).

NEUROSCIENCE

"Flash and freeze" synaptic plasticity

The eponymous mossy fibers in the hippocampus of the brain connect the dentate gyrus to the CA3 pyramidal region and are implicated in short-term memories spanning a few seconds to minutes. How such memories are held in the circuit is a puzzle. One candidate is post-tetanic potentiation, a form of synaptic plasticity that decays within tens to hundreds of seconds and regulates synaptic strength at hippocampal mossy fiber–CA3 pyramidal neuron synapses. Vandael *et al*. simultaneously recorded from pairs of mossy fiber terminals and postsynaptic CA3 pyramidal neurons and followed measurements with "flash and freeze" electron microscopy. The authors observed that post-tetanic potentiation was induced by an increase in the size of the readily releasable pool of synaptic vesicles in granule cells. —PRS

Neuron 10.1016/j.neuron.2020.05.013 (2020).

QUANTUM INFORMATION

Set, exchange, measure, repeat

Successful protocols for quantum information processing and quantum computation depend on the reliable storage and manipulation of the quantum state of a qubit. Qubits, however, are prone to errors because complete isolation from the environment is not possible. Methods for correcting these errors must also contend with the fact that direct measurement of a qubit destroys it. Xue *et al*. describe a repetitive quantum nondemolition method on a two-qubit system in which the state of the main qubit is mapped onto a second qubit that acts as an ancilla. Repeated measurement of the ancilla qubit allows the main qubit to be maintained and read out with higher fidelity. Such a protocol provides a route to robust quantum information processing in solid-state systems. —ISO

Phys. Rev. X **10**, 021006 (2020).

SCIENCE AND POLICY

Testing peer review in government

To improve the role of science in government, some have called for more peer review. To test this approach, Ho and Larrimore Ouellette randomly assigned pending U.S. patent applications to external academic technical experts whose reviews were then provided to government patent examiners. Compared with matched, non–peer-reviewed applications, peer review increased examiners' efforts to search for earlier publications ("prior art"), increased citations to nonpatent literature, and reduced the propensity to initially grant the application. However, the effort was costly because of strict requirements (for example, time spent translating legalese) for how reviews could be introduced into the examination process. —BW

J. Empir. Leg. Stud. **17**, 190 (2020).

PHOTO: DAVID L. MOORE - AK/ALAMY STOCK PHOTO

REVIEW SUMMARY

FOREST AND CLIMATE

Climate-driven risks to the climate mitigation potential of forests

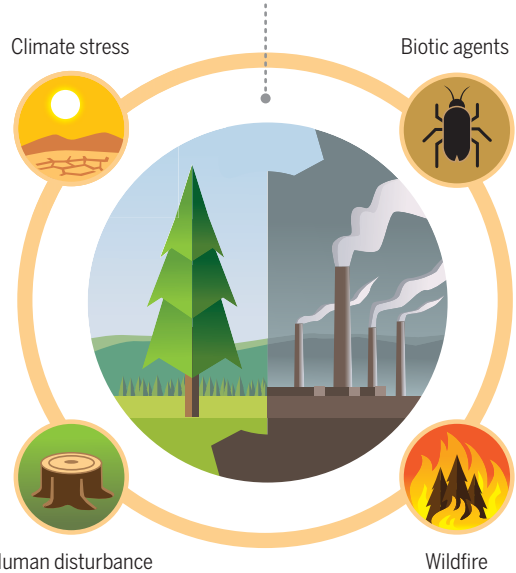
William R. L. Anderegg*, Anna T. Trugman, Grayson Badgley, Christa M. Anderson, Ann Bartuska, Philippe Ciais, Danny Cullenward, Christopher B. Field, Jeremy Freeman, Scott J. Goetz, Jeffrey A. Hicke, Deborah Huntzinger, Robert B. Jackson, John Nickerson, Stephen Pacala, James T. Randerson

BACKGROUND: Forests have considerable potential to help mitigate human-caused climate change and provide society with a broad range of cobenefits. Local, national, and international efforts have developed policies and economic incentives to protect and enhance forest carbon sinks—ranging from the Bonn Challenge to restore deforested areas to the development of forest carbon offset projects around the world. However, these policies do not always account for important ecological and climate-related risks and limits to forest stability (i.e., permanence). Widespread climate-induced forest die-off has been observed in forests globally and creates a dangerous carbon cycle feedback, both by releasing large amounts of carbon stored in forest ecosystems to the atmosphere and by reducing the size of the future forest carbon sink. Climate-driven risks may fundamentally compromise forest carbon stocks and sinks in the 21st century. Understanding and quantifying climate-driven risks to forest stability are crucial components needed to forecast the integrity of forest carbon sinks and the extent to which they can contribute toward the Paris Agreement goal to limit warming well below 2°C. Thus, rigorous scientific assessment of the risks and limitations to widespread deployment of forests as natural climate solutions is urgently needed.

ADVANCES: Many forest-based natural climate solutions do not yet rely on the best available scientific information and ecological tools to assess the risks to forest stability from climate-driven forest dieback caused by fire, drought, biotic agents, and other disturbances. Crucially, many of these permanence risks are projected to increase in the 21st century because of climate change, and thus estimates based on historical data will underestimate the true risks that forests face. Forest climate policy needs to fully account for the permanence risks because they could fundamentally undermine the effectiveness of forest-based climate solutions.

Here, we synthesize current scientific understanding of the climate-driven risks to forests and highlight key issues for maximizing the effectiveness of forests as natural climate solutions. We lay out a roadmap for quantifying current and forecasting future risks to forest stability using recent advances in vegetation physiology, disturbance ecology, mechanistic vegetation modeling, large-scale ecological observation networks, and remote sensing. Finally, we review current efforts to use forests as natural climate solutions and discuss how these programs and policies presently consider and could more fully embrace physiological, climatic, and permanence uncertainty about the future of forest carbon stores and the terrestrial carbon sink.

Forests as natural climate solutions face fundamental limits and underappreciated risks



Effective use of forests as natural climate solutions depends on accounting for climate-driven risks, such as fire and drought. Leveraging cutting-edge scientific tools holds great promise for improving and guiding the use of forests as natural climate solutions, both in estimating the potential of carbon storage and in estimating the risks to forest carbon storage.

OUTLOOK: The scientific community agrees that forests can contribute to global efforts to mitigate human-caused climate change. The community also recognizes that using forests as natural climate solutions must not distract from rapid reductions in emissions from fossil fuel combustion. Furthermore, responsibly using forests as natural climate solutions requires rigorous quantification of risks to forest stability, forests' carbon storage potential, cobenefits for species conservation and ecosystem services, and full climate feedbacks from albedo and other effects. Combining long-term satellite records with forest plot data can provide rigorous, spatially explicit estimates of climate change–driven stresses and disturbances that decrease productivity and increase mortality. Current vegetation models also hold substantial promise to quantify forest risks and inform forest management and policies, which currently rely predominantly on historical data.

A more-holistic understanding and quantification of risks to forest stability will help policy-makers effectively use forests as natural climate solutions. Scientific advances have increased our ability to characterize risks associated with a number of biotic and abiotic factors, including risks associated with fire, drought, and biotic agent outbreaks. While the models that are used to predict disturbance risks of these types represent the cutting edge in ecology and Earth system science to date, relatively little infrastructure and few tools have been developed to interface between scientists and foresters, land managers, and policy-makers to ensure that science-based risks and opportunities are fully accounted for in policy and management contexts. To enable effective policy and management decisions, these tools must be openly accessible, transparent, modular, applicable across scales, and usable by a wide range of stakeholders. Strengthening this science-policy link is a critical next step in moving forward with leveraging forests in climate change mitigation efforts. ■

ON OUR WEBSITE

Read the full article at https://dx.doi.org/10.1126/science.aaz7005

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ILLUSTRATION: DAVID MEIKLE

RESEARCH ARTICLE SUMMARY

SOLAR CELLS

Gas chromatography–mass spectrometry analyses of encapsulated stable perovskite solar cells

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INTRODUCTION: Although advances in materials and processing have led to remarkable advancements in the energy conversion efficiency of perovskite solar cells (PSCs), increasing from 3.8% to 25.2% in only 10 years, these solar cells cannot become commercially viable unless their underperforming durability is improved. The instability of perovskites must be addressed if PSCs are to compete with silicon technology, which currently offers a 25-year performance warranty. Previous approaches to this problem include the use of metal oxide barrier layers and butyl rubber sealants. Here, we report a low-cost polymer/glass stack encapsulation scheme that enables PSCs to pass the demanding International Electrotechnical Commission (IEC) 61215:2016 Damp Heat and Humidity Freeze tests. These tests help to determine whether solar cell modules can withstand the effects of outdoor operating conditions by exposing them to repeated temperature cycling (–40° to 85°C) as well as 85% relative humidity. Our airtight encapsulation scheme prevented moisture ingress. It was also effective in suppressing outgassing of decomposition products, which limits decomposition reactions of organic hybrid PSCs by allowing these reactions to come to equilibrium. The gas compositions were verified by gas chromatography–mass spectrometry (GC-MS).

RATIONALE: In the GC-MS technique, gas chromatography separates the components in a mixture, and the chemical identity of each component is determined with mass spectrometry. We could directly identify with high specificity the decomposition products of multi-cation perovskite precursors, of unencapsulated perovskite test structures, and of encapsulated full cells at elevated temperatures. The results allowed us to identify thermal degradation pathways by determining the outgassing products of mixed-cation perovskites during heating. We then used GC-MS to evaluate the effectiveness of different packaging techniques developed for PSCs. The packaging schemes were a polyisobutylene (PIB)–based polymer blanket encapsulation, a polyolefin-based blanket encapsulation, and a PIB edge seal. These packaging layers were then capped by a glass cover. For the edge seal, the decomposition gases inside the cell were sampled with a syringe. The feasibilities of these packaging techniques were also demonstrated by IEC photovoltaic module standard Damp Heat and Humidity Freeze testing.

RESULTS: Signature decomposition products such as CH_3I, CH_3Br, and NH_3 were identified and decomposition pathways were proposed for CH_3NH_3I (MAI), $HC(NH_2)_2I$ (FAI), CH_3NH_3Br (MABr), and mixed-cation and mixed-halide $(FAI)_{0.85} + (MABr)_{0.15}$ perovskite precursors, including their secondary decomposition reactions at 350°, 140°, and 85°C. The GC-MS results confirmed that the Br-containing precursor was less prone to thermal decomposition than an I-containing precursor. Also, CsFAMA cells were found to outgas one-fifth as much decomposition product as their FAMA counterparts, which indicated that the Cs-containing cells had better thermal stability. Although the decomposition of FAI is reversible, the mixing of MA with FA precursors caused decomposition products to participate in the secondary reaction that was irreversible. This finding confirmed the disadvantage of mixing of MA with FA perovskite through the reduction in chemical stability. The blanket-encapsulated PSCs sustained no efficiency degradation after 1800 hours of Damp Heat testing or 75 cycles of Humidity Freeze testing.

CONCLUSION: GC-MS identified signature volatile products of the decomposition of organic hybrid perovskites under thermal stress, thereby informing decomposition pathways. The findings are important for developing potential cell-stabilizing strategies, given that cells in the field typically experience high operating temperatures. In addition, results of GC-MS confirm that the low-cost pressure-tight encapsulation we developed is effective in suppressing such outgassing and therefore decomposition reactions of PSCs. This encapsulation scheme is the simplest of all for perovskite cells to pass IEC photovoltaic module standard tests. Our approach can be applied to evaluating the effectiveness of other packaging approaches, as well as testing the effectiveness of coatings and material compositions aimed at limiting light and thermal degradation. ■

ON OUR WEBSITE

Read the full article at https://dx.doi.org/10.1126/science.aba2412

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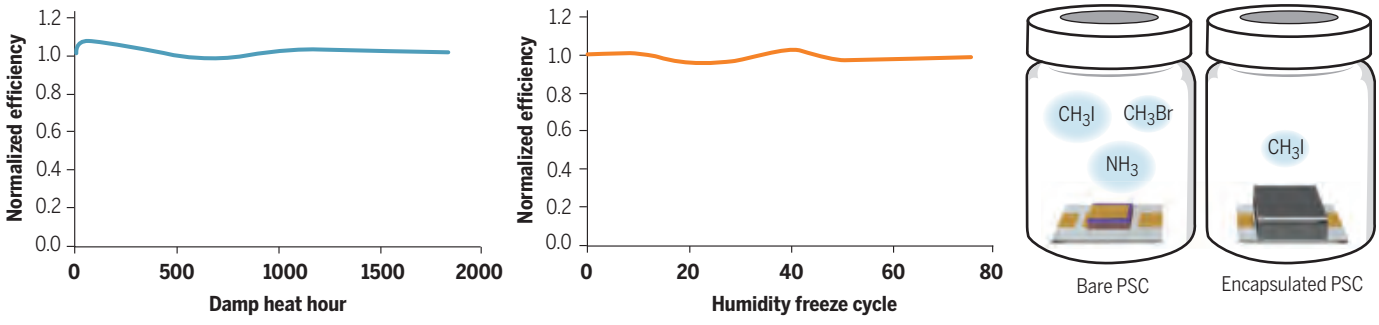
Cite this article as L. Shi *et al.*, *Science* **368**, eaba2412 (2020). DOI: 10.1126/science.aba2412



Stable perovskite solar cells exceeding the requirements of the IEC 61215 Damp Heat and Humidity Freeze tests. Unencapsulated and encapsulated perovskite cells were analyzed by gas chromatography–mass spectrometry, detecting signature volatile products of the organic hybrid perovskite decomposition under thermal stress and confirming the effectiveness of the low-cost pressure-tight polymer/glass stack encapsulation schemes developed.

RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

Manipulating synthetic optogenetic odors reveals the coding logic of olfactory perception

Edmund Chong*, Monica Moroni*, Christopher Wilson, Shy Shoham, Stefano Panzeri, Dmitry Rinberg†

INTRODUCTION: Advances in monitoring brain activity at large and fine scales have revealed tremendous complexity in how the brain responds to, and represents, the external world. Although many features in brain activity patterns (which brain cells fire and when) are found to correlate with changes in the external sensory world, it is not yet known which activity features are consequential for perception and how they are combined to generate percepts. Some studies have shown that many of these correlated changes in activity may be redundant or even epiphenomenal.

RATIONALE: To address how brain activity generates perception, we directly and systematically manipulated neural activity in the mouse olfactory system while measuring perceptual responses. Mouse olfaction is an attractive model system because the relevant brain circuitry has already been carefully mapped out and is accessible for direct manipulation. We used genetically engineered mice in which brain cells can be activated simply by shining light on them—a technique known as optogenetics. Optogenetics allowed us to directly generate and manipulate brain activity in a precise and parametric manner. We first trained mice to recognize light-driven activity patterns in the olfactory system, or "synthetic odors." Subsequently, we measured how recognition changed as we systematically manipulated learned activity patterns. Some manipulations led to larger changes in recognition than others, and the degree of change reflected the importance of each manipulated feature to perception. By the additional manipulation of multiple features simultaneously, we could precisely quantify how individual features combined to produce perception.

RESULTS: The perceptual responses of mice not only depended on which groups of cells were activated, but also on their activation latencies, i.e., temporal sequences akin to timed notes in a melody. Critically, the most perceptually relevant activation latencies were defined relative to other cells in a sequence and not to brain or body rhythms (e.g., animal sniffing) as previously hypothesized from observational studies. Moreover, earlier-activated cells in the sequence had a larger effect on behavioral responses; modifying later cells in the sequence had small effects. To account for all results, we formulated a simple computational model based on template matching, in which new activity sequences are compared with learned sequences or templates. The model weighs relative timing within each sequence and also accounts for the greater importance of earlier-activated cells. Based on our model, the degree of mismatch between the new sequence and learned template predicts the extent to which recognition should degrade as neural activity changes across many different manipulations.

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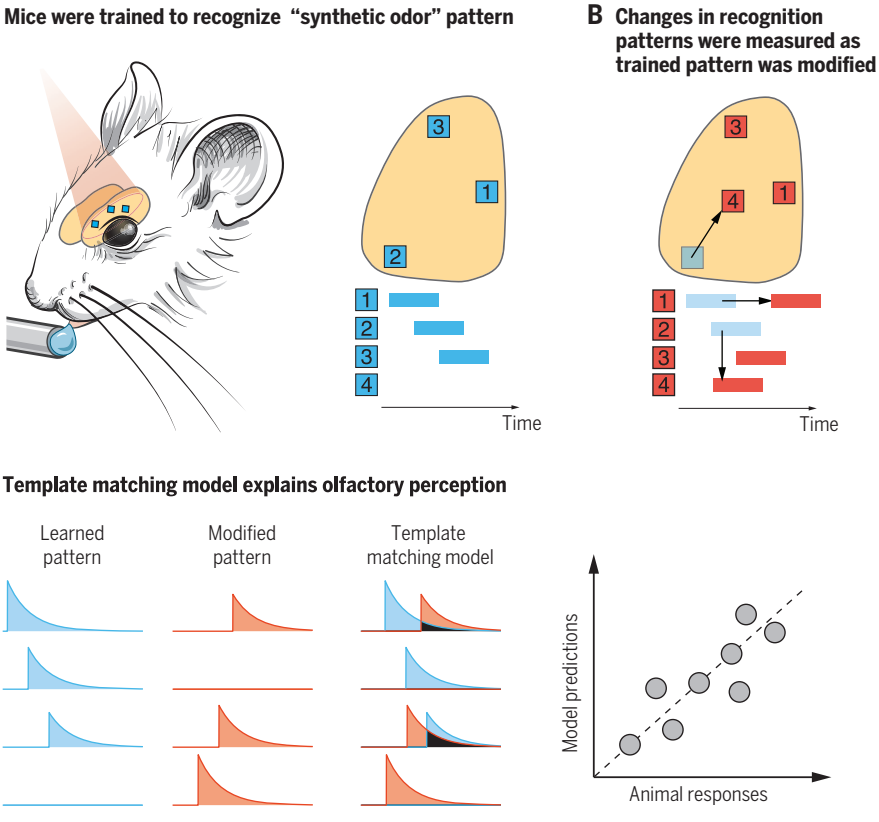
CONCLUSION: We developed an experimental and theoretical framework to map a broad space of precisely and systematically manipulated brain activity patterns to behavioral responses. Using this framework, we uncovered key computations made by the olfactory system on neural activity to generate percepts and derived a systematic model of olfactory processing directly relevant for perception. Our framework forms a powerful, general approach for causally testing the links between brain activity and perception or behavior. This framework is especially pertinent given the continued development of advanced tools for manipulating brain activity at fine scales across various brain regions. ■


A Mice were trained to recognize "synthetic odor" pattern
B Changes in recognition patterns were measured as trained pattern was modified
C Template matching model explains olfactory perception
Learned pattern
Modified pattern
Template matching model
Time
Time
Model predictions
Animal responses

Probing olfactory perception with synthetic odors. (**A**) We trained mice to recognize synthetic odor patterns: artificially stimulated neural activity in the olfactory bulb. Patterns were defined in space (top right) and time (bottom right). (**B**) Perceptual responses were measured across systematic modifications of trained patterns. (**C**) Template-matching model of pattern activity (left) accounts for perceptual responses (right).

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Cite this article as E. Chong *et al.*, *Science* **368**, eaba2357 (2020). DOI: 10.1126/science.aba2357

RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

Meissner corpuscles and their spatially intermingled afferents underlie gentle touch perception

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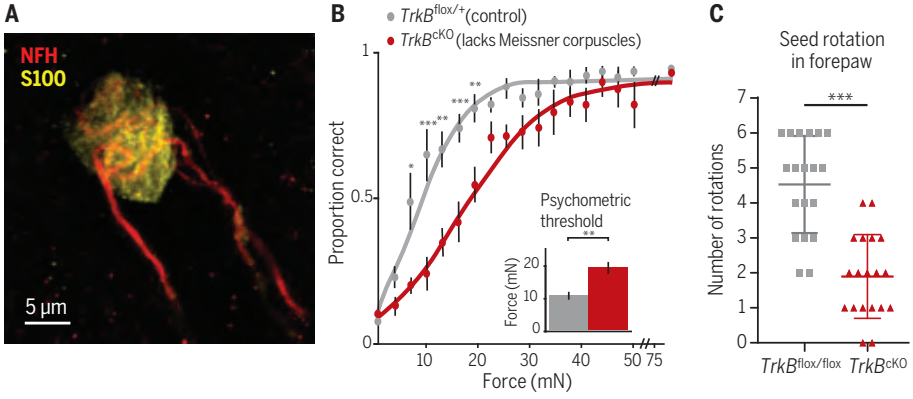
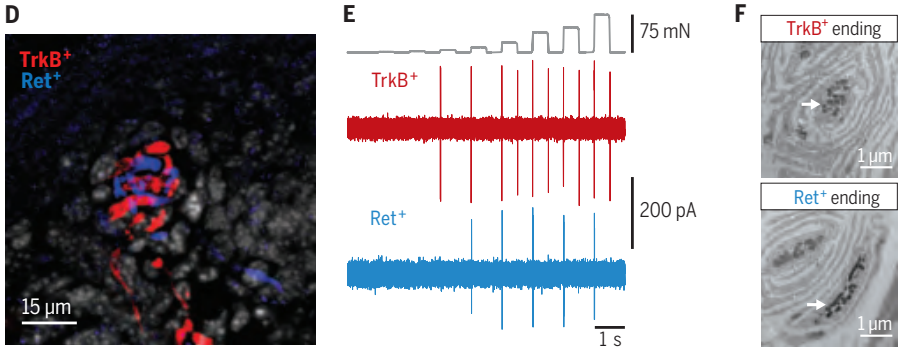
INTRODUCTION: Meissner corpuscles are mechanosensory end organs that densely occupy mammalian glabrous skin. The basic anatomy of the Meissner corpuscle and the Aβ (large soma diameter and fast action potential conducting) mechanosensory neurons that innervate it have been widely described. However, little is known about the requirement for the Meissner corpuscle and its innervating Aβ mechanosensory neurons for touch-related behaviors, sensorimotor capabilities, and tactile perception.

RATIONALE: Mice lacking either brain-derived neurotrophic factor (BDNF) or its receptor TrkB have a range of developmental deficits, including an absence of Meissner corpuscles. We reasoned that confining TrkB manipulations to sensory neurons in conjunction with selective genetic labeling approaches would allow us to investigate the developmental assembly of Meissner corpuscles and their functions in somatosensation. While examining Meissner corpuscle development, we found that two Aβ mechanosensory neuron types innervate the corpuscle. Here, we assessed the requirement of Meissner corpuscles for touch sensitivity and fine sensorimotor control and the anatomical, physiological, and ultrastructural properties of the two Aβ sensory neurons that innervate this mechanosensory end organ.

Meissner corpuscles are required for perception of gentle touch and fine sensorimotor control



Two molecularly and physiologically distinct Aβ neurons innervate Meissner corpuscles and differentially associate with corpuscle lamellar cells



Anatomy and physiology of Meissner corpuscles required for tactile behavior. (**A** to **C**) Meissner corpuscles and their Aβ afferents (A) were required for normal tactile sensitivity (B) and fine sensorimotor control (C). (**D**) TrkB$^+$ and Ret$^+$ Aβ neurons both innervated Meissner corpuscles. (**E** and **F**) TrkB$^+$ Meissner afferents were more sensitive (E) and had more lamellar cell wrappings [(F), arrows indicate axons wrapped with lamellar processes] compared with Ret$^+$ Meissner afferents. In (A), S100 and NFH are antibodies used to visualize Meissner corpuscles and their afferents, respectively. In (B) and (C), error bars represent SEM, and *p < 0.05, **p < 0.01, and ***p < 0.001.

RESULTS: Sensory neuron–specific knockout of TrkB resulted in complete loss of Meissner corpuscles without affecting other mechanosensory end organs in mouse glabrous (nonhairy) skin, including Aβ mechanosensory neuron–Merkel cell complexes and Pacinian corpuscles. Behavioral measurements showed that mice lacking Meissner corpuscles were deficient in perceiving and reacting to the gentlest detectable forces acting on glabrous skin and in fine sensorimotor control.

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Read the full article at https://dx.doi.org/10.1126/science.abb2751

Genetic labeling experiments revealed that Meissner corpuscles are innervated by two molecularly distinct Aβ sensory neuron types, one that expresses TrkB and the other that expresses the tyrosine kinase Ret. Despite innervating the same end organ, the responses of these two Aβ neuron types to tactile stimuli differed: The TrkB-positive (TrkB$^+$) Meissner afferent was more sensitive and responded at both the onset and the offset of step indentations of glabrous skin, whereas the Ret$^+$ Meissner afferent was less sensitive and rarely responded at step offset. Some Ret$^+$ neurons even had sustained responses during a static indentation stimulus. In addition, the axonal endings of these two Aβ mechanosensory neuron types were found to be homotypically tiled but heterotypically offset. Computational modeling suggested that this anatomical arrangement maximizes information available for encoding acuity while ensuring complete coverage of the skin using a limited number of neurons. Finally, ultrastructural analysis using electron microscopy revealed that the axon terminals of the more sensitive TrkB$^+$ Meissner afferents had greater numbers of lamellar cell wrappings than the terminals of the less sensitive Ret$^+$ Meissner afferents.

CONCLUSION: We conclude that two homotypically tiled but heterotypically offset Aβ mechanosensory neurons with distinct molecular, physiological, and ultrastructural properties innervate Meissner corpuscles, which underlie the perception of, and behavioral responses to, the gentlest detectable forces acting on glabrous skin. ■

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Cite this article as N. L. Neubarth *et al.*, *Science* 368, eabb2751 (2020). DOI: 10.1126/science.abb2751

RESEARCH ARTICLES

CORONAVIRUS

Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease

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SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is the etiological agent responsible for the global COVID-19 (coronavirus disease 2019) outbreak. The main protease of SARS-CoV-2, M^{pro}, is a key enzyme that plays a pivotal role in mediating viral replication and transcription. We designed and synthesized two lead compounds (11a and 11b) targeting M^{pro}. Both exhibited excellent inhibitory activity and potent anti–SARS-CoV-2 infection activity. The x-ray crystal structures of SARS-CoV-2 M^{pro} in complex with 11a or 11b, both determined at a resolution of 1.5 angstroms, showed that the aldehyde groups of 11a and 11b are covalently bound to cysteine 145 of M^{pro}. Both compounds showed good pharmacokinetic properties in vivo, and 11a also exhibited low toxicity, which suggests that these compounds are promising drug candidates.

In late December 2019, a cluster of pneumonia cases caused by a novel coronavirus was reported in Wuhan, China (*1*–*3*). Genomic sequencing showed that this pathogenic coronavirus is 96% identical to a bat coronavirus and shares 79.6% sequence identity to SARS-CoV (*4*–*6*). This novel coronavirus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses, and the pneumonia was designated as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) on 11 February 2020 (*7*). The epidemic spread rapidly to more than 212 countries and was announced as a global health emergency by WHO (*8*). No clinically effective vaccines or specific antiviral drugs are currently available for the prevention and treatment of COVID-19 infections. The combination of α-interferon and the anti-HIV drugs lopinavir/ritonavir (Kaletra) has been used, but the curative effect remains very limited and there can be toxic side effects (*9*). Remdesivir, a broad-spectrum antiviral drug developed by Gilead Sciences Inc., is also being explored for the treatment of COVID-19, but more data are needed to prove its efficacy (*10*–*12*). Specific anti–SARS-CoV-2 drugs offering efficacy and safety are urgently needed.

A maximum likelihood tree based on the genomic sequence showed that the virus falls within the subgenus *Sarbecovirus* of the genus *Betacoronavirus* (*6*). Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses. The genomic RNA of coronaviruses is ~30,000 nucleotides in length with a 5′-cap structure and a 3′-poly(A) tail, and contains at least six open reading frames (ORFs) (*13*, *14*). The first ORF (ORF 1a/b), about two-thirds of the genome length, directly translates two polyproteins, pp1a and pp1ab, so named because there is an a-1 frameshift between ORF1a and ORF1b. These polyproteins are processed by a main protease, M^{pro} [also known as the 3C-like protease ($3CL^{pro}$)], and by one or two papain-like proteases, into 16 nonstructural proteins (NSPs). These NSPs engage in the production of subgenomic RNAs that encode four main structural proteins [envelope (E), membrane (M), spike (S), and nucleocapsid (N) proteins] and other accessory proteins (*15*, *16*). Therefore,

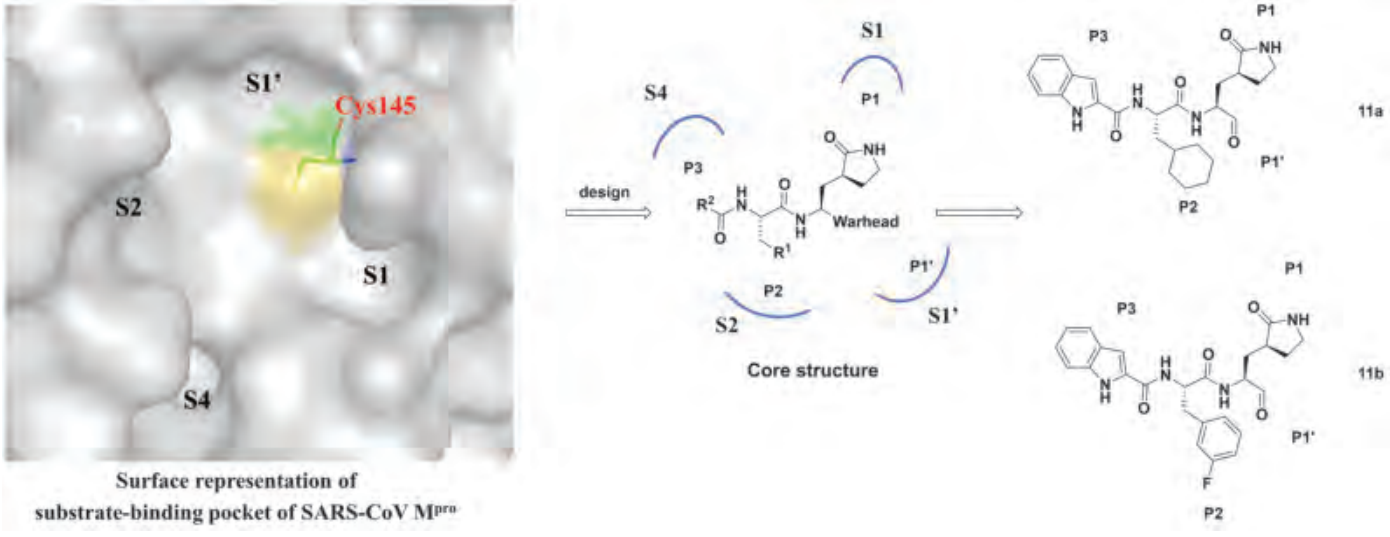

Fig. 1. Design strategy of SARS-CoV-2 main protease (M^{pro}) inhibitors and the chemical structures of 11a and 11b.

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these proteases, especially M^{pro}, play a vital role in the life cycle of coronaviruses.

M^{pro} is a three-domain (domains I to III) cysteine protease involved in most maturation cleavage events within the precursor polyprotein (*17*–*19*). Active M^{pro} is a homodimer containing two protomers. The coronavirus M^{pro} features a noncanonical Cys-His dyad located in the cleft between domains I and II (*17*–*19*). M^{pro} is conserved among coronaviruses, and several common features are shared among the substrates of M^{pro} in different coronaviruses. The amino acids in substrates from the N terminus to the C terminus are numbered as follows: -P4-P3-P2-P1↓P1′-P2′-P3′-, with the cleavage site between P1 and P1′. In particular, a Gln is almost always required in the P1 position of the substrates. Because M^{pro} has no human homolog, it is an ideal antiviral target (*20*–*22*).

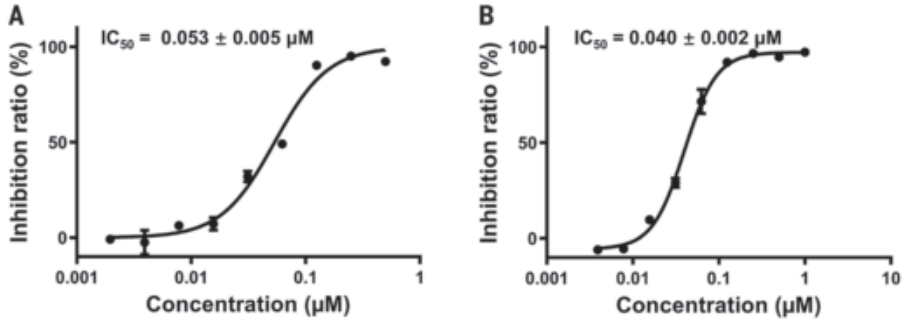

Fig. 2. Inhibitory activity profiles of compounds 11a (A) and 11b (B) against SARS-CoV-2 M^{pro}.

Design and synthesis of 11a and 11b

The active sites of M^{pro} are highly conserved among all coronavirus M^{pro}s and are usually composed of four sites: S1′, S1, S2, and S4 (*22*). We were able to design and synthesize inhibitors targeting SARS-CoV-2 M^{pro} by analyzing the substrate-binding pocket of SARS-CoV M^{pro} (PDB ID 2H2Z) (Fig. 1). The thiol of a cysteine residue in the S1′ site anchors inhibitors by a covalent linkage that is important for the inhibitors to maintain antiviral activity. In our design of new inhibitors, an aldehyde was selected as a new warhead in P1 in order to form a covalent bond with cysteine. The reported SARS-CoV M^{pro} inhibitors often have an (*S*)-γ-lactam ring that occupies the S1 site of M^{pro}, and this ring was expected to be a good choice in P1 (*23*). Furthermore, the S2 site of coronavirus M^{pro} is usually large enough to accommodate the larger P2 fragment. To test

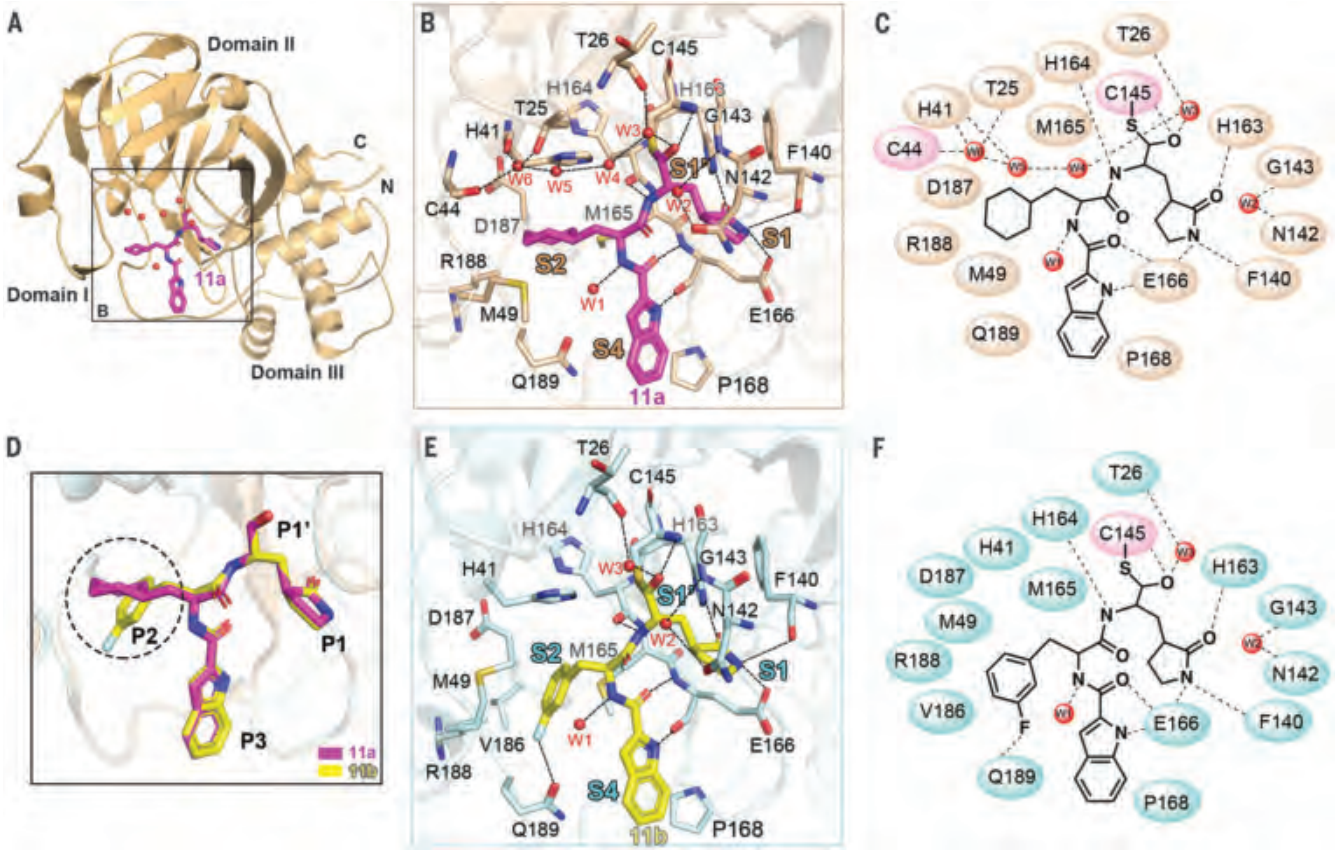

Fig. 3. M^{pro}-inhibitor binding modes for 11a and 11b. (**A**) Cartoon representation of the crystal structure of SARS-CoV-2 M^{pro} in complex with **11a**. Compound **11a** is shown as magenta sticks; water molecules are shown as red spheres. (**B**) Close-up view of the **11a** binding pocket. Four subsites, S1′, S1, S2, and S4, are labeled. The residues involved in inhibitor binding are shown as wheat sticks. **11a** and water molecules are shown as magenta sticks and red spheres, respectively. Hydrogen bonds are indicated as dashed lines. (**C**) Schematic diagram of SARS-CoV-2 M^{pro}–**11a** interactions shown in (B). (**D**) Comparison of the binding modes of **11a** and **11b** in SARS-CoV-2 M^{pro}. The major difference between **11a** and **11b** is marked with a dashed circle; **11a** and **11b** are shown as magenta and yellow sticks, respectively. (**E**) Close-up view of the **11b** binding pocket. Hydrogen bonds are indicated as dashed lines. (**F**) Schematic diagram of SARS-CoV-2 M^{pro}–**11b** interactions shown in (E). Amino acid abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

the importance of different ring systems, we introduced a cyclohexyl or 3-fluorophenyl into P2, with the fluorine expected to enhance activity. An indole group was introduced into P3 to form new hydrogen bonds with S4 and improve drug-like properties.

The synthetic route and chemical structures of the compounds (**11a** and **11b**) are shown in scheme S1. The starting material (*N*-Boc-L-glutamic acid dimethyl ester **1**) was obtained from commercial suppliers and used without further purification to synthesize the key intermediate ***3*** according to the literature (*24*). The intermediates **6a** and **6b** were synthesized from **4** and acids **5a** and **5b**. Removal of the *t*-butoxycarbonyl group from **6a** and **6b** yielded **7a** and **7b**. Coupling **7a** and **7b** with the acid **8** yielded the esters **9a** and **9b**. The peptidomimetic aldehydes **11a** and **11b** were approached through a two-step route in which the ester derivatives **9** were first reduced with $NaBH_4$ to generate the primary alcohols **10a** and **10b**, which were subsequently oxidized into aldehydes **11a** and **11b** with Dess-Martin periodinane (DMP).

11a and 11b are potent inhibitors of M^{pro}

Recombinant SARS-CoV-2 M^{pro} was expressed and purified from *Escherichia coli* (*18*, *25*). A fluorescently labeled substrate—MCA-AVLQ↓SGFR-Lys(Dnp)-Lys-NH_2, derived from the N-terminal autocleavage sequence from the viral protease—was designed and synthesized for the enzymatic assay.

Both **11a** and **11b** exhibited high SARS-CoV-2 M^{pro} inhibition activity, which reached 100% for **11a** and 96% for **11b** at 1 μM, respectively. We used a fluorescence resonance energy transfer (FRET)–based cleavage assay to determine the median inhibitory concentration (IC_{50}) values. The results revealed excellent inhibitory potency, with IC_{50} values of 0.053 ± 0.005 μM and 0.040 ± 0.002 μM for **11a** and **11b**, respectively (Fig. 2).

Structures of SARS-CoV-2 M^{pro} in complex with 11a and 11b

To elucidate the mechanism of inhibition of SARS-CoV-2 M^{pro} by **11a**, we determined the crystal structure of this complex at 1.5 Å resolution (table S1). The crystal of M^{pro}-**11a** belongs to the space group *C*2, and an asymmetric unit contains only one molecule (table S1). Two molecules (designated protomer A and protomer B) associate into a homodimer around a crystallographic two-fold symmetry axis (fig. S2). The structure of each protomer contains three domains with the substrate-binding site located in the cleft between domains I and II. At the active site of SARS-CoV-2 M^{pro}, Cys^{145} and His^{41} (Cys-His) form a catalytic dyad (fig. S2).

The electron density map clearly shows compound **11a** in the substrate-binding pocket of SARS-CoV-2 M^{pro} in an extended conformation (Fig. 3A and fig. S3, A and B). Details of the interaction are shown in Fig. 3, B and C. The electron density shows that the C of the aldehyde group of **11a** and the catalytic-site Cys^{145} of SARS-CoV-2 M^{pro} form a standard 1.8 Å C–S covalent bond. The oxygen atom of the aldehyde group also plays a crucial role in stabilizing the conformations of the inhibitor by forming a 2.9 Å hydrogen bond with the backbone of residue Cys^{145} in the S1′ site. The (*S*)-γ-lactam ring of **11a** at P1 fits well into the S1 site. The oxygen of the (*S*)-γ-lactam group

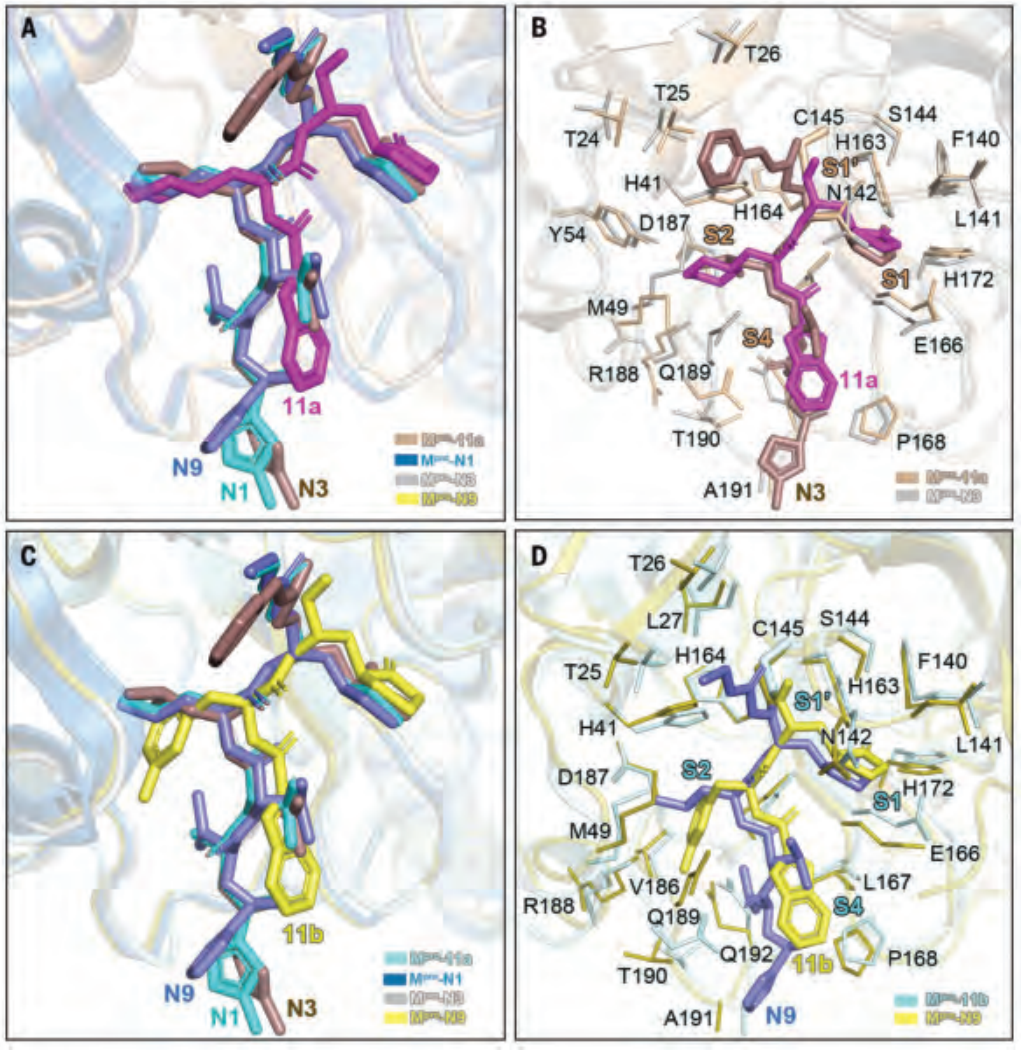

Fig. 4. Comparison of the inhibitor binding modes in SARS-CoV M^{pro} and SARS-CoV-2 M^{pro}. (**A**) Comparison of binding modes of **11a** in SARS-CoV-2 M^{pro} with those of **N1**, **N3**, and **N9** in SARS-CoV M^{pro}. SARS-CoV-2 M^{pro}–**11a** (wheat; PDB code 6LZE), SARS-CoV M^{pro}–**N1** (sky blue; PDB code 1WOF), SARS-CoV M^{pro}–**N3** (gray; PDB code 2AMQ), and SARS-CoV M^{pro}–**N9** (olive; PDB code 2AMD) are shown as cartoons. **11a**, **N1**, **N3**, and **N9** are shown in magenta, cyan, dirty violet, and salt, respectively. (**B**) Comparison of the **11a** and **N3** binding pockets. Residues in the M^{pro}-**11a** and M^{pro}-**N3** structures are colored in wheat and gray, respectively. **11a** and **N3** are shown as sticks colored in magenta and dirty violet, respectively. (**C**) Comparison of binding modes of **11b** in SARS-CoV-2 M^{pro} with those of **N1**, **N3**, and **N9** in SARS-CoV M^{pro}. SARS-CoV-2 M^{pro}–**11b** (PDB code 6M0K) is shown as a pale cyan cartoon. **11b**, **N1**, **N3**, and **N9** are shown in yellow, cyan, dirty violet, and salt, respectively. (**D**) Comparison of the **11b** and **N9** binding pockets. Residues in the M^{pro}-**11b** and M^{pro}-**N9** structures are colored in pale cyan and olive, respectively. **11b** and **N9** are shown as sticks colored in yellow and salt, respectively.

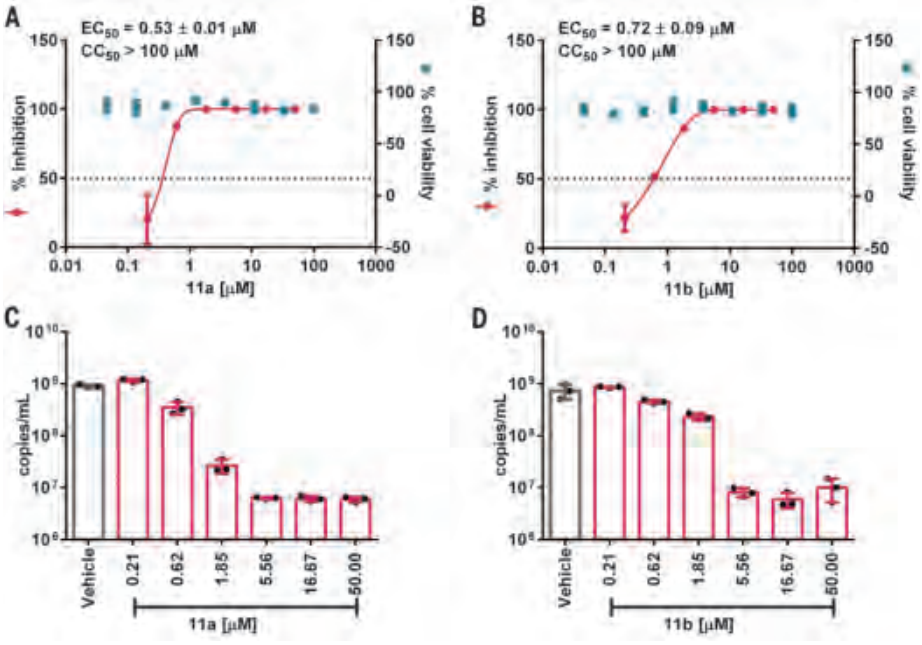

Fig. 5. In vitro inhibition of viral main protease inhibitors against SARS-CoV-2. (**A** and **B**) Vero E6 cells were treated with a series concentration of indicated compounds **11a** and **11b** and infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.05. At 24 hours after infection, viral yield in the cell supernatant was quantified by plaque assay. The cytotoxicity of these compounds in Vero E6 cells was also determined by using CCK8 assays. The left and right *y* axis of each graph represents mean percent inhibition of virus yield and mean percent cell viability of the drugs, respectively. (**C** and **D**) Viral RNA copy numbers in the cell supernatants were quantified by qRT-PCR. Data are means ± SD; *n* = 3 biological replicates.

forms a 2.7 Å hydrogen bond with the side chain of His^{163}. The main chain of Phe^{140} and side chain of Glu^{166} also participate in stabilizing the (*S*)-γ-lactam ring by forming 3.2 Å and 3.0 Å hydrogen bonds with its NH group, respectively. In addition, the amide bonds on the chain of **11a** form hydrogen bonds with the main chains of His^{164} (3.2 Å) and Glu^{166} (2.8 Å), respectively. The cyclohexyl moiety of **11a** at P2 deeply inserts into the S2 site, stacking with the imidazole ring of His^{41}. The cyclohexyl group is also surrounded by the side chains of Met^{49}, Tyr^{54}, Met^{165}, Asp^{187}, and Arg^{188}, producing extensive hydrophobic interactions. The indole group of **11a** at P3 is exposed to solvent (S4 site) and is stabilized by Glu^{166} through a 2.6 Å hydrogen bond. The side chains of residues Pro^{168} and Gln^{189} interact with the indole group of **11a** through hydrophobic interactions. Multiple water molecules (named W1 to W6) play an important role in binding **11a**. W1 interacts with the amide bonds of **11a** through a 2.9 Å hydrogen bond, whereas W2 to W6 contribute to stabilizing **11a** in the binding pocket by forming a number of hydrogen bonds with the aldehyde group of **11a** and the residues of Asn^{142}, Gly^{143}, Thr^{26}, Thr^{25}, His^{41}, and Cys^{44}.

The crystal structure of SARS-CoV-2 M^{pro} in complex with **11b** is very similar to that of the **11a** complex and shows a similar inhibitor-binding mode (Fig. 3D and figs. S3, C and D, and S4A). The difference in binding mode is most probably due to the 3-fluorophenyl group of **11b** at P2. Relative to the cyclohexyl group in **11a**, the 3-fluorophenyl group undergoes a downward rotation (Fig. 3D). The side chains of residues His^{41}, Met^{49}, Met^{165}, Val^{186}, Asp^{187}, and Arg^{188} interact with this aryl group through hydrophobic interactions, and the side chain of Gln^{189} stabilizes the 3-fluorophenyl group with an additional 3.0 Å hydrogen bond (Fig. 3, E and F). In short, these two crystal structures reveal a similar inhibitory mechanism in which both compounds occupy the substrate-binding pocket and block the enzyme activity of SARS-CoV-2 M^{pro}.

Compounds **N1**, **N3**, and **N9** are wide-spectrum inhibitors targeting coronavirus M^{pro}. Compared with the binding modes of **N1**, **N3**, and **N9** in SARS-CoV M^{pro} complex structures reported previously, the binding modes of **11a** and **11b** in SARS-CoV-2 M^{pro} complex structures are similar and the differences among these overall structures are small (Fig. 4 and fig. S4, B to F) (*22*). The differences mainly lie in the interactions at S1′, S2, and S4 subsites, possibly because the sizes of functional groups vary at the corresponding P1′, P2, and P4 sites in the inhibitors (Fig. 4, A and C).

Antiviral activity of 11a and 11b

To further substantiate the enzyme inhibition results, we evaluated the ability of these compounds to inhibit SARS-CoV-2 in vitro (Fig. 5 and fig. S5). As shown in Fig. 5, compounds **11a** and **11b** exhibited good anti–SARS-CoV-2 infection activity in cell culture, with half-maximal effective concentration (EC_{50}) values of 0.53 ± 0.01 μM and 0.72 ± 0.09 μM, respectively, by plaque assay. Neither compound caused cytotoxicity, with half cytotoxic concentration (CC_{50}) values of >100 μM, yielding selectivity indices for **11a** and **11b** of >189 and >139, respectively. We also used immunofluorescence and quantitative real-time polymerase chain reaction (qRT-PCR) to monitor the antiviral activity of **11a** and **11b**. The results showed that **11a** and **11b** exhibit a good antiviral effect on SARS-CoV-2 (Fig. 5 and fig. S5).

Pharmacokinetic and toxicity studies

To explore the further druggability of the compounds **11a** and **11b**, we evaluated both compounds for their pharmacokinetic properties. As shown in table S2, compound **11a** given to mice intraperitoneally (5 mg/kg) and intravenously (5 mg/kg) displayed a half-life ($T_{1/2}$) of 4.27 hours and 4.41 hours, respectively, and we observed a high maximal concentration (C_{max} = 2394 ng/ml) and a good bioavailability of 87.8% when the compound **11a** was given intraperitoneally. Metabolic stability of **11a** in mice was also good (clearance = 17.4 ml min^{-1} kg^{-1}). When administered intraperitoneally (20 mg/kg), subcutaneously (5 mg/kg), and intravenously (5 mg/kg), compound **11b** also showed good pharmacokinetic properties (its bioavailability exceeded 80% when given both intraperitoneally and subcutaneously, and it displayed a longer $T_{1/2}$ of 5.21 hours when given intraperitoneally). Considering the danger of COVID-19, we selected intravenous drip administration for further study because of its comparatively high area under the curve (AUC) value and rapid effect. Relative to **11a** administrated intravenously in CD-1 mice, **11b** displayed a shorter $T_{1/2}$ (1.65 hours) and a faster clearance rate (clearance = 20.6 ml min^{-1} kg^{-1}). Compound **11a** was selected for further investigation with intravenous drip dosing in Sprague-Dawley (SD) rats and beagle dogs. The results showed (table S3) that **11a** exhibited long $T_{1/2}$ values (SD rat, 7.6 hours; beagle dog, 5.5 hours), low clearance rates (rat, 4.01 ml min^{-1} kg^{-1}; dog, 5.8 ml min^{-1} kg^{-1}), and high AUC values (rat, 41,500 hours·ng/ml; dog, 14,900 hours·ng/ml). The above pharmacokinetic results indicate that compound **11a** warrants further study.

An in vivo toxicity study (table S4) of **11a** was carried out on SD rats and beagle dogs. The acute toxicity of **11a** was measured in SD rats. No SD rats died after receiving 40 mg/kg by intravenous drip administration. When the dosage was raised to 60 mg/kg, one of four SD rats died. The dose range toxicity of **11a** was studied for 7 days at dosing levels of 2, 6, and 18 mg/kg in SD rats and at 10 to 40 mg/kg in

beagle dogs. All animals received once-daily (QD) dosing by intravenous drip, and all animals were clinically observed at least once a day. No obvious toxicity was observed in either group. These data indicate that **11a** is a good candidate for further clinical study.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6497/1331/suppl/DC1
Materials and Methods
Scheme S1
Figs. S1 to S5
Tables S1 to S4
References (*26*–*29*)

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INFLUENZA

Different genetic barriers for resistance to HA stem antibodies in influenza H3 and H1 viruses

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The discovery and characterization of broadly neutralizing human antibodies (bnAbs) to the highly conserved stem region of influenza hemagglutinin (HA) have contributed to considerations of a universal influenza vaccine. However, the potential for resistance to stem bnAbs also needs to be more thoroughly evaluated. Using deep mutational scanning, with a focus on epitope residues, we found that the genetic barrier to resistance to stem bnAbs is low for the H3 subtype but substantially higher for the H1 subtype owing to structural differences in the HA stem. Several strong resistance mutations in H3 can be observed in naturally circulating strains and do not reduce in vitro viral fitness and in vivo pathogenicity. This study highlights a potential challenge for development of a truly universal influenza vaccine.

The major surface antigen of influenza virus, the hemagglutinin (HA), is composed of a highly variable globular head domain that contains the receptor binding site and a highly conserved stem domain that contains the membrane fusion machinery (*1*). The major antigenic sites on HA are located on the globular head (*2*, *3*), which is immunodominant over the stem (*4*). However, most antibodies to the head domain are strain specific, whereas antibodies to the stem are harder to elicit but have considerably more breadth [reviewed in (*5*)]. The isolation, characterization, and structure determination of broadly neutralizing human antibodies (bnAbs) to the HA stem since 2008 (*6*–*9*) have provided insights into immunogen design toward a universal influenza vaccine (*8*, *10*–*13*) and have offered templates for design of small proteins, peptides, and small molecules against influenza virus (*14*–*18*). Several stem bnAbs are also currently in clinical trials (*19*). Therefore, stem bnAbs open up multiple promising avenues to tackle this challenging global health problem.

Resistance mutations can be a major obstacle for antiviral and vaccine development, but the extent to which this is a problem for stem bnAbs is unclear. Several studies have reported difficulty in selecting strong resistance mutations to stem bnAbs even after extensive virus passaging (*20*–*22*), or through deep mutational scanning (*23*), which is a comprehensive and unbiased approach (*24*). Nonetheless, other studies have obtained strong resistance mutations through virus passaging (*6*, *21*, *25*). Here, we systematically compare the extent to which resistance can emerge to stem bnAbs in HAs from the H3 and H1 subtypes that represent the circulating influenza A strains.

Deep mutational scanning of the major HA stem epitope in H3/HK68 HA

CR9114 (*26*) and FI6v3 (*27*) are two prototypic bnAbs that bind the HA stem and represent those with the greatest breadth. Both FI6v3 and CR9114 neutralize group 1 and 2 influenza A viruses (*26*, *27*), and CR9114 further cross-reacts with influenza B HA (*26*). Deep mutational scanning (*24*), which combines saturation mutagenesis and next-generation sequencing, has been applied to study how HA mutations affect influenza viral fitness (*28*–*30*) and to identify viral mutants that are resistant

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to antibodies against HA (*31*). Here, we used deep mutational scanning of the HA stem of H3N2 A/Hong Kong/1/1968 (H3/HK68) to search for virus resistance mutations to CR9114 and FI6v3. Unlike previous studies that examined the entire HA (*28*–*31*), we focused on eight residues in HA2 (HA0 is cleaved during maturation into HA1 and HA2) of H3/HK68 HA in and around the main stem epitope (the region recognized by an antibody): namely Q42, I45, D46, Q47, I48, N49, L52, and T111 (Fig. 1, A to C). All except T111 are located on HA2 helix A, which is a common target for stem bnAbs. Residues 42, 45, 46, 48, 49, and 52 were chosen because they interact with CR9114 and FI6v3, and residue 47 because it interacts with FI6v3 (Fig. 1, D and E). Completely buried T111 was selected because its mutation in H5 HA enabled escape from CR6261 (*6*), which binds an epitope similar to CR9114 (*9*, *26*).

By considering mutations to all amino acids at each position, we quantified the in vitro viral fitness of 147 of 152 possible single mutants and 6234 of 10,108 possible double mutants across the eight residues of interest in H3/HK68 under five conditions: no antibody, 2 μg/ml CR9114 immunoglobulin G (IgG), 10 μg/ml CR9114 IgG, 0.3 μg/ml FI6v3 IgG, and 2.5 μg/ml FI6v3 IgG (fig. S1). Many mutants have a relative fitness [proxy for replication fitness (*30*)] similar to that of the wild type (WT), which was set as 1 (Fig. 2, A and B), indicating that the HA stem region can tolerate many mutations.

We further quantified the relative resistance (normalized to WT) of each viral mutant by measuring relative fitness (normalized to WT) with or without antibody (fig. S2). Many resistance mutations to CR9114 and FI6v3 were observed (Fig. 2, C and D), and most were located at HA2 residues 42 and 45, which are important in the binding interface with CR9114 and FI6v3 (Fig. 1, D and E). The double mutants also showed high relative resistance if one mutation exhibited high relative resistance even if the other did not (fig. S3A). Consistently, the relative resistance of double mutants can generally be explained by additivity of their corresponding single mutants (fig. S3B). These results demonstrate the prevalence of H3/HK68 resistance mutations to stem bnAbs and identify cross-resistance mutations to both CR9114 and FI6v3, which are encoded by different germline genes and have very different angles of approach to the HA (*5*).

Validation of resistance mutations

We then individually constructed and tested 18 single and 6 double HA mutants of the

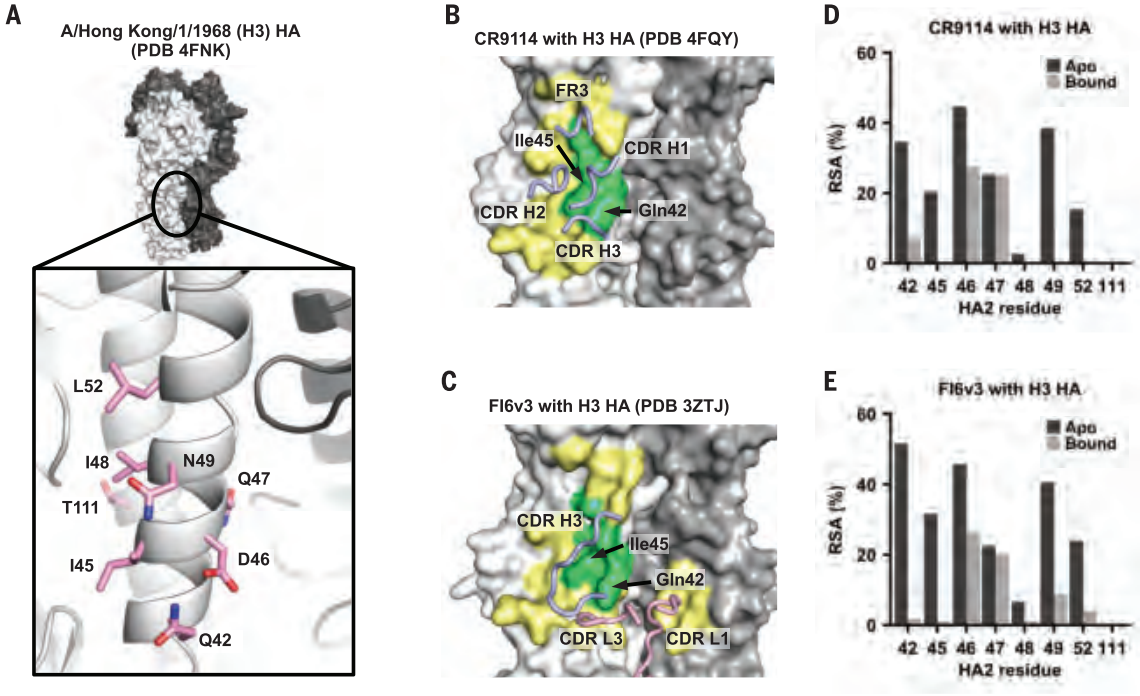

Fig. 1. Epitopes of broadly neutralizing antibodies to the HA stem. (**A**) The location of residues of interest in this study on the HA structures. All residues of interest are on HA2. One protomer of the trimer is shown in light gray and the other two protomers in dark gray, and a detailed view of the location of the residues of interest is shown in the inset. (**B** and **C**) Epitopes of (B) CR9114 Fab in complex with H3 HA (PDB 4FQY) (*26*) and (C) FI6v3 in complex with H3 HA (PDB 3ZTJ) (*27*) are colored in yellow and green, with residues of interest colored in green. The arrows indicate the positions of HA2 residues 42 and 45, which are in the center of the bnAb epitopes. Antibody paratopes (CDRs and FR regions) are shown in tube representation and labeled accordingly. Blue, heavy chain; pink, light chain. (**D** and **E**) The relative solvent accessibility (RSA) of each residue of interest is shown. Black bar, apo form; gray bar, Fab-bound form.

Table 1. Binding affinity of IgGs and Fabs against HAs of WT and different H3/HK68 HA2 mutants. n.b., no binding.

K_d (nM)	WT	HA2-I45T	HA2-I45M	HA2-I45F	HA2-N49T
CR9114 Fab	43.2 ± 0.4	779.6 ± 50.0	319.1 ± 12.1	n.b.	11.3 ± 0.4
27F3 Fab	122.6 ± 3.0	n.b.	n.b.	n.b.	105.0 ± 1.0
CR9114 IgG	<0.1	164.8 ± 2.3	75.0 ± 1.0	n.b.	<0.1
27F3 IgG	1.7 ± 0.1	n.b.	n.b.	n.b.	1.9 ± 0.9
FI6v3 IgG	<0.1	7.8 ± 0.2	6.7 ± 0.1	62.0 ± 0.2	<0.1
S139/1 IgG	2.1 ± 0.1	3.1 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	2.8 ± 0.1

H3/HK68 virus spanning a range of relative resistance against CR9114 and FI6v3 IgGs (Fig. 3A). The minimum inhibitory concentration (MIC) in a microneutralization assay strongly correlated with the relative resistance from deep mutational scanning (Spearman's rank correlation > 0.8, Fig. 3B), with several mutants showing strong cross-resistance to both CR9114 and FI6v3. For example, the MICs of CR9114 and FI6v3 against mutants I45Y/S/N/F/W were all >100 and ≥20 μg/ml, respectively, compared to 3.1 and 0.2 μg/ml for WT. This validation experiment substantiates our finding that strong resistance mutations are prevalent in H3/HK68.

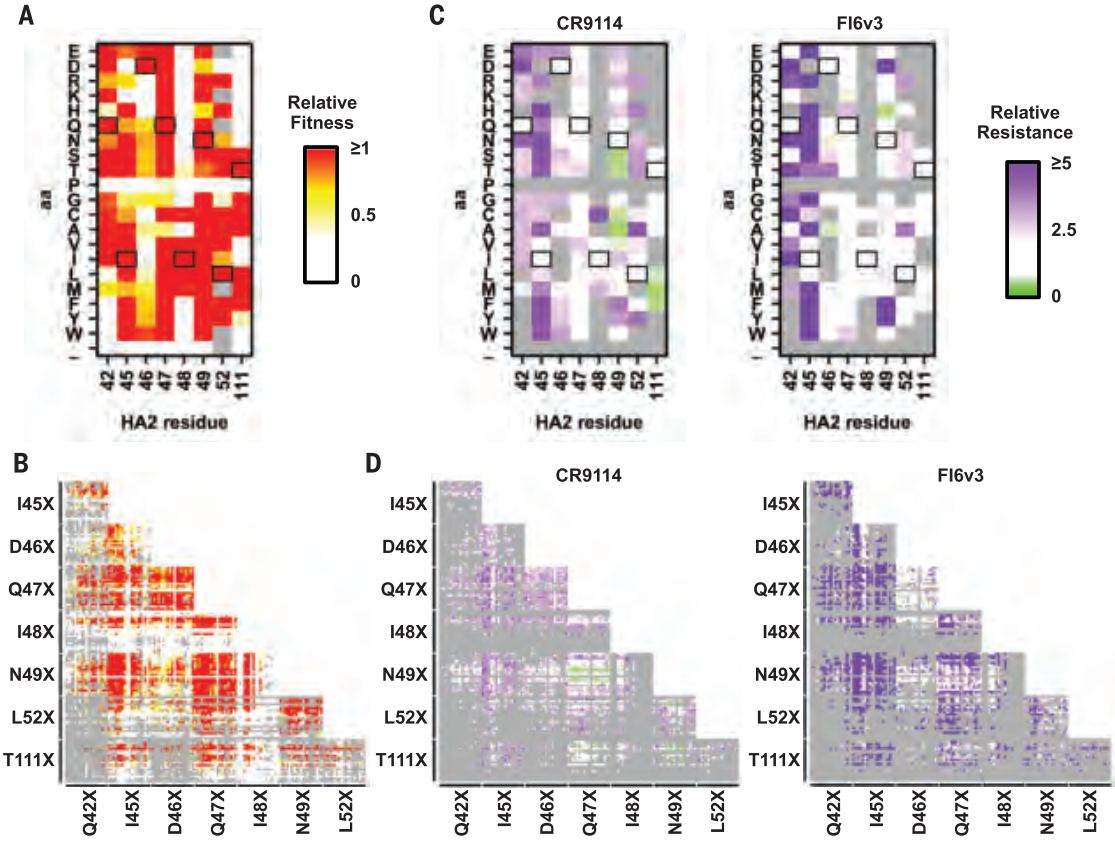

Fig. 2. Fitness and resistance profile of H3/HK68 HA2 single and double viral mutants. (**A** and **B**) On the basis of the deep mutational scanning experiment, the relative fitness of (A) single and (B) double mutants is shown with WT set as 1. In (A) and (B), mutants with a next-generation sequencing read count of less than 20 from the plasmid mutant library are excluded and shown as gray. (**C** and **D**) Relative resistance for (C) each single and (D) each double mutant against 10 μg/ml CR9114 antibody or 2.5 μg/ml FI6v3 antibody is shown. Relative resistance for WT is set as 1. In (C) and (D), mutants with a relative fitness of less than 0.5 are shown as gray. Residues that correspond to the WT sequence are boxed. In (B) and (D), each color point in the heatmap represents a double mutant. Of note, some mutants with an increased sensitivity to antibody are shown in green on the relative resistance color scale. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

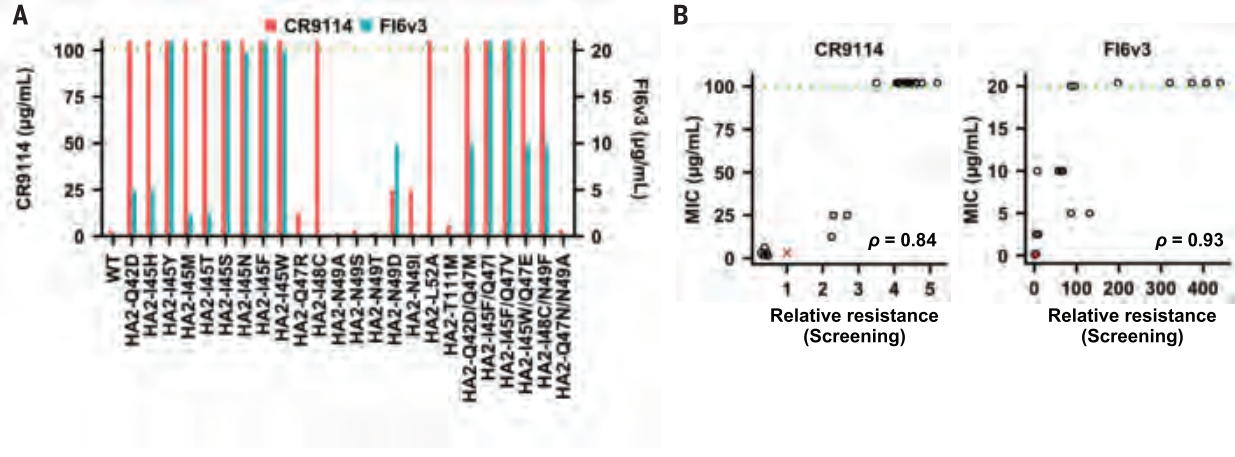

Fig. 3. Characterization of antibody-resistant mutants. (**A**) The minimum inhibitory concentration (MIC) of CR9114 and FI6v3 to individual viral mutants is shown. The MIC of CR9114 is in red and represented by the *y* axis on the left. The MIC of FI6v3 is in blue and represented by the *y* axis on the right. (**B**) The Spearman's rank correlations (ρ) between the MIC measured from individual mutants and the relative resistance (against 10 μg/ml CR9114 antibody or 2.5 μg/ml FI6v3 antibody) computed from the deep mutational scanning experiment (screening) are shown. The green dashed line represents the upper detection limit in (A) and (B). Wild type is represented by the red "X".

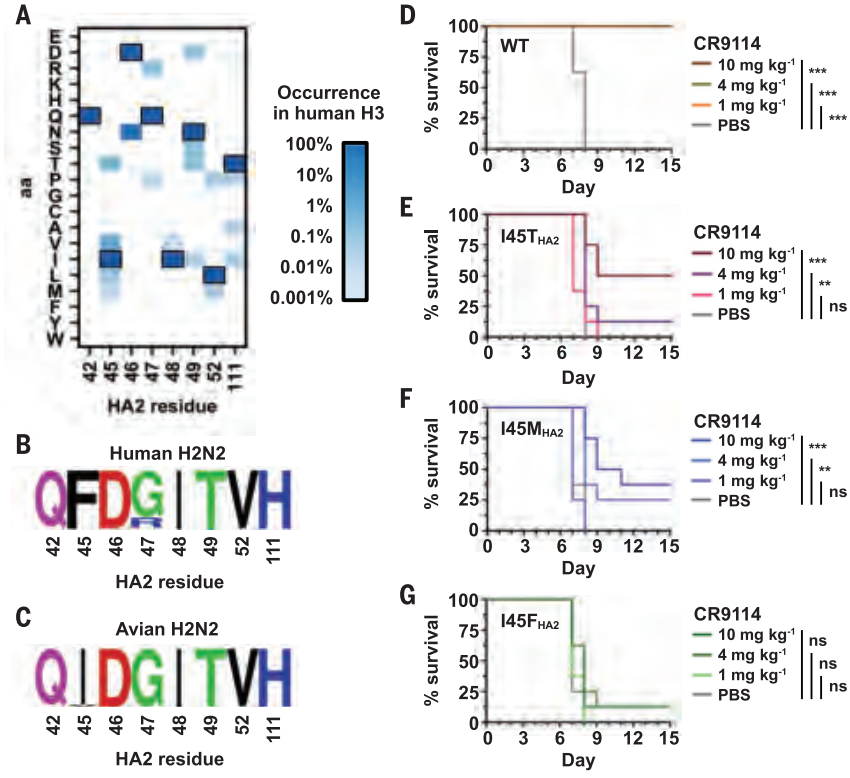

Fig. 4. In vivo characterization and natural occurrence of antibody-resistant mutants. (**A**) The natural occurrence frequencies of different amino acid variants at the residues of interest in human H3 HAs are shown as a heatmap. (**B** and **C**) The natural occurrence frequencies of different amino acid variants at the residues of interest in (B) human H2N2 HA or (C) avian H2N2 HA are shown as sequence logos. (**D** to **G**) Prophylactic protection experiments were performed with different doses of CR9114 against (D) WT, (E) HA2 I45T mutant, (F) HA2 I45M mutant, and (G) HA2 I45F mutant. Recombinant H3/HK68 (7:1 on H1/PR8 backbone) viruses were used. Lethal doses [25 median lethal dose (MLD_{50})] of WT or mutant viruses were used. Kaplan-Meier survival curves are shown. Paired analysis of each treatment group, relative to control, was conducted using Log-rank (Mantel-Cox) tests. ***$P \leq 0.001$; **$P \leq 0.01$; ns (not significant), $P > 0.05$.

Natural occurrence of resistance mutations

Next, we explored whether these resistance mutations can be found in naturally circulating strains and identified a few of these strong resistance mutations at low frequency in human H3N2 isolates (*32*), including I45T, I45M, and N49D (Fig. 4A), although most have not been observed yet in naturally circulating strains. I45T is also observed in human H3N2 isolates sequenced without any passaging (fig. S4A), implying that its presence is not due to a passaging artifact (*33*). Moreover, strong cross-resistance mutation I45F was found in all human H2N2 viruses that circulated from 1957 to 1968 (Fig. 4B and fig. S4B), whereas almost all avian H2N2 viruses have Ile^{45} (Fig. 4C). This particular mutation in human H2N2 viruses explains why it is more difficult for some stem bnAbs to bind or neutralize this subtype compared to other subtypes, as found in previous studies (*6*, *26*, *34*). Thus, resistance mutations to stem bnAbs already do indeed occur in circulating strains.

In vivo pathogenicity and escape of resistance mutations

Given their relevance to circulating strains, we further tested the in vivo viral pathogenicity of HA2 mutants I45T, I45M, and I45F. The weight loss profiles in mice after infection by HA2 mutant and WT viruses were comparable (fig. S5), indicating that these resistance mutations do not reduce in vivo pathogenicity. We further demonstrated that I45T, I45M, and I45F could escape in vivo prophylactic protection. Whereas mice infected with WT were completely protected by CR9114 IgG at all tested doses (1, 4, and 10 mg/kg), mutants I45T, I45M, and I45F were lethal even at the highest dose of CR9114 IgG (Fig. 4, D to G).

Resistance mutations decrease affinity to bnAbs

To dissect the resistance mechanism, we tested binding of H3/HK68 I45T, I45M, and I45F recombinant HAs to CR9114 and FI6v3, and to another stem bnAb, 27F3 (*34*), which uses the same V_H1-69 germ line as CR9114 and similarly neutralizes group 1 and 2 influenza A viruses. Binding (dissociation constant K_d) of CR9114 Fab, CR9114 IgG, 27F3 Fab, 27F3 IgG, and FI6v3 IgG to the HA mutants (Table 1 and fig. S6) was diminished, particularly with I45F, where binding to CR9114 and 27F3 (Fab and IgG) was undetectable. By contrast, binding of these stem Fabs and IgGs to the HA mutant N49T, which did not exhibit resistance against CR9114 and FI6v3 (Figs. 2C and 3A), was comparable to that of the WT (Table 1). As a control, we also tested binding of bnAb S139/1 that targets the receptor binding site far from the stem epitope (*35*, *36*). S139/1 IgG affinities for those HA mutants (K_d = 1.8 to 3.1 nM) were similar to that of the WT (K_d = 2.1 nM). Thus, virus resistance to stem bnAbs correlated with a decrease in binding affinity for the mutant HAs.

To evaluate the structural basis of the resistance, we determined crystal structures of HAs with HA2 mutations I45T, I45M, and I45F to 2.1 to 2.5 Å resolutions (table S1 and fig. S7A). Compared to WT (Ile^{45}), the shorter side chain of I45T would create a void when CR9114 is bound (fig. S7B) that would be energetically unfavorable. By contrast, the longer flexible side chain of I45M would likely clash with CR9114 (fig. S7B), but CR9114 can still bind the I45M mutant, albeit with much lower affinity than WT (Table 1). The I45F mutant, however, would clash more severely with CR9114, and no binding was detected (Table 1 and fig. S7B). Similar observations for FI6v3 (fig. S7C) explain the sensitivity of CR9114 and FI6v3 to mutations at HA2 residue 45.

Resistance to HA stem bnAbs is subtype specific

We further examined whether mutations that conferred strong resistance in H3/HK68 would have the same phenotypes in other H3 strains. Consequently, we examined I45T, I45M, and I45F mutations on H3N2 A/Wuhan/359/95 (H3/Wuhan95) virus. These mutants had WT-like titers after viral rescue and passaging (fig. S8A), showed a plaque size similar to that of WT (fig. S8B), conferred strong resistance to FI6v3 (fig. S8, C and D), and, therefore, have similar phenotypes in H3/HK68 and H3/Wuhan95 viruses. This result led us to hypothesize that strong resistance mutants to stem bnAbs are readily attainable in human H3 strains that span a wide range of years, as well as explore whether the same phenomenon can be observed in the H1 subtype, which is the other currently circulating influenza A subtype in the human population.

In contrast to our findings for H3/HK68, deep mutational scanning found that resistance mutants of H1N1 A/WSN/33 (H1/WSN) virus to FI6v3 were rare and had only very small effects (*23*). To further investigate the difference between H3 and H1 strains, we performed four additional deep mutational scanning experiments—three with H1N1 strains, namely A/Solomon Islands/3/2006 (H1/SI06) against FI6v3, A/Michigan/45/2015 (H1/Mich15) against FI6v3, and H1/WSN against CR9114; and one with H3N2 strain A/Perth/16/2009 (H3/Perth09) against FI6v3. The H1/SI06 and H1/Mich15 HA mutant virus libraries contain all possible single substitutions at HA2 residues 42, 45, 46, 47, 48, 49, 52, and 111, whereas H1/WSN and H3/Perth09 HA mutant virus libraries both contain all possible single substitutions across the entire HA and were constructed previously (*37*, *38*). We also analyzed the previously published dataset on H1/WSN against FI6v3 (*23*).

To compare H1/SI06, H1/Mich15, H1/WSN, and H3/Perth09 to H3/HK68, we computed the relative resistance of mutations at HA2 residues 42, 45, 46, 47, 48, 49, 52, and 111 (Fig. 5, A to E). Similar to H3/HK68, resistance mutations are highly prevalent in H3/Perth09 (Fig. 5C). Conversely, resistance mutations were rare in H1/SI06, H1/Mich15, and H1/WSN (Fig. 5, A to E). We further calculated the fraction surviving (*23*) for each viral mutant across the entire H1/WSN and H3/Perth09 HAs during antibody selection. Fraction surviving is a quantitative measure for the resistance and is normalized across deep mutational scanning experiments (*23*). For example, fraction surviving values of 1, 0.5, and 0.1 indicate that the replication fitness in the presence of antibody selection is 100, 50, and 10% of that without antibody selection. The fraction surviving values of H1/WSN mutants against CR9114 were all very small, similar to previous observations of H1/WSN against FI6v3 (Fig. 5F and fig. S10). In stark contrast, many mutants of H3/Perth09 were identified with a large fraction surviving value (Fig. 5F). Consistent with the relative resistance profile of H3/HK68 (Fig. 2C), a number of H3/Perth09 mutants with a large fraction surviving value were found at HA2 residues 42 and 45 (Fig. 5F and fig. S11A). Moreover, mutations at HA2 residue 53, which were not examined in H3/HK68 (Fig. 2), had high fraction surviving values in H3/Perth09 against FI6v3 (fig. S11, A and B). In H3 HA, mutation of HA2 residue 53 would abolish a hydrogen bond to FI6v3 (fig. S11B). Together, these results suggest that the prevalence of resistance mutations to stem bnAbs is a general phenomenon for the H3 subtype but not the H1 subtype.

Subtype-specific differences in the HA stem

We next aimed to elucidate the mechanism that underlies the lower genetic barrier to

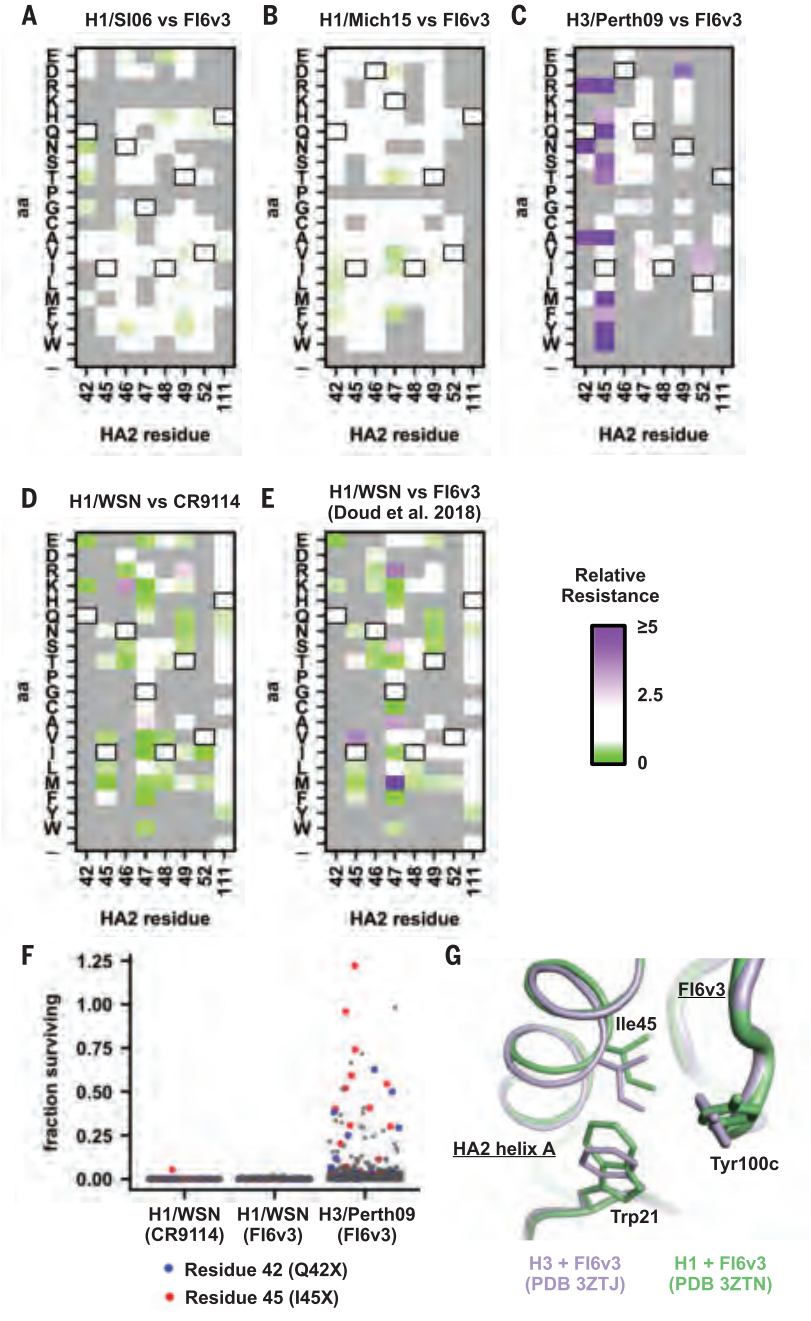

Fig. 5. Relative resistance profile of single mutants in multiple strains of influenza virus. (**A** to **E**) Relative resistance is measured for each single viral mutant at HA2 residues 42, 45, 46, 47, 48, 49, 52, and 111 of (A) H1/SI06 against 300 ng/ml FI6v3 antibody, (**B**) H1/Mich15 against 300 ng/ml FI6v3 antibody, (**C**) H1/WSN against 100 ng/ml CR9114 antibody, (**D**) H1/WSN against 200 ng/ml FI6v3 antibody [data are from (*23*)], and (**E**) H3/Perth09 against 15 μg/ml FI6v3 antibody. Relative resistance for WT is set as 1. Mutants with a relative fitness of less than 0.5 are excluded and shown as gray. Residues that correspond to the WT sequence are boxed. (**F**) Fraction surviving for all single mutants across the HA protein is shown. Each data point represents one mutant. Fraction surviving was computed as previously described (*23*). Assuming no antibody-mediated enhancement of virus replication, the theoretical upper limit for the fraction surviving is 1, which indicates that the replication fitness is the same with and without antibody selection (in practice, fraction surviving values slightly >1 can sometimes be obtained because of the experimental error in quantitative polymerase chain reaction and next-generation sequencing). Data for H1/WSN against FI6v3 were from a previous study (*23*). Data points that represent mutations at residues 42 and 45 are colored in blue and red, respectively, and mutations at other residues are in gray. (**G**) The crystal structures of H3 HA in complex with FI6v3 (PDB 3ZTJ) (*27*) and H1 HA in complex with FI6v3 (PDB 3ZTN) (*27*) were compared by aligning their heavy-chain variable domains. Because the structure of CR9114 with H1 HA is not available, a similar analysis was not performed on CR9114.

resistance to stem bnAbs in H3 HA as compared to H1 HA. Many mutations at HA2 residue 45 have a high fitness cost in H1/SI06 (fig. S9A), which can increase the genetic barrier to resistance. However, most mutations at HA2 residue 45 have no fitness cost in H1/Mich15. In addition, the mutational fitness profiles of H1/SI06 and H1/Mich15 (fig. S9, A and B) show that many mutations can be tolerated in the HA stem, similar to H3/HK68 (Fig. 2A and fig. S9, C to F). Thus, the difference in genetic barrier to resistance to stem bnAbs between H1 and H3 subtypes cannot be fully explained by their ability to tolerate mutations (i.e., fitness cost of mutations).

We therefore further compared the structures of FI6v3 in complexes with H1 HA and H3 HA (*27*). The orientation of Tyr100c [Kabat numbering (*39*)] on the third complementarity determining region of the antibody heavy chain (CDR H3) of FI6v3 differs when binding to H1 or H3 HAs (Fig. 5G). The position of HA2 Ile^{45} also differs between H1 and H3 HAs when FI6v3 is bound. As a result, Tyr100c of FI6v3 packs tighter to HA2 Ile^{45} of H3 than of H1 HA. Thus, a bulkier substitution at residue 45 of HA2 will disrupt binding of FI6v3 to H3 HA more than to H1 HA. Therefore, the low genetic barrier to resistance to stem bnAbs in the H3 subtype can be partly attributed to both high mutational tolerance in the HA stem and subtype-specific structural features, but it is likely that the latter plays a more critical role in determining subtype-specific differences. Although a number of subtype-specific structural features are known in the stem region (*40*), how they influence the genetic barriers for resistance to stem bnAbs remains to be addressed in future studies.

Implications for escape from a universal vaccine or therapeutic stem bnAbs

Prior studies of influenza bnAbs have not considered whether different subtypes might have different abilities to generate resistance mutations against bnAbs. We find here that H3 HA has a much lower genetic barrier to resistance to two of the broadest bnAbs, CR9114 and FI6v3, as compared to H1 HA, consistent with reports of strong resistance to other human HA stem antibodies in the H3 subtype (*21*, *25*, *41*) versus none (*21*) to weak resistance (*22*, *23*) in the H1 subtype. Therefore, it may be easier for stem bnAbs to suppress H1 rather than H3 subtype viruses.

Because the HA stem is immunosubdominant to the head domain, immunological pressure on the HA stem may not yet be sufficient to affect the evolution of circulating human viruses (*42*). However, several stem bnAbs are currently in clinical trials (*19*), and some recently developed influenza vaccine immunogens have focused on targeting the HA stem (*5*, *8*). If stem bnAbs begin to be distributed on a global scale, the immunological pressure on the HA stem will increase. Our findings here indicate that resistance mutations could emerge, at least in the H3 subtype.

Although resistance mutations to stem bnAbs are still rare in currently circulating influenza strains (Fig. 4A), it is important to evaluate the potential impact of such mutations, because many vaccine strategies aim to elicit anti-stem antibodies. We could not overcome some key resistance mutations (I45T, I45M, and I45F) by in vitro evolution of CR9114 (fig. S12). Nonetheless, the best strategy to prevent or overcome such resistance may involve delivery or elicitation of a combination of antibodies with different resistance profiles. The discovery and characterization of bnAbs with different escape profiles will continue to be key to broadening our arsenal against influenza virus. For example, human H2N2 virus, which carries a Phe at HA2 residue 45, often has low reactivity with stem bnAbs (*6*, *26*, *27*, *34*, *43*), although a few can have high potency against human H2N2 (*44*–*46*). Future studies on anti-stem responses against human H2N2 and emerging viruses, such as H5N1 and H7N9, may provide further insights into how to overcome potential resistance should immune pressure become focused on the HA stem.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6497/1335/suppl/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 to S3
References (*50*–*69*)

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BIODIVERSITY CHANGE

Landscape-scale forest loss as a catalyst of population and biodiversity change

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Global biodiversity assessments have highlighted land-use change as a key driver of biodiversity change. However, there is little empirical evidence of how habitat transformations such as forest loss and gain are reshaping biodiversity over time. We quantified how change in forest cover has influenced temporal shifts in populations and ecological assemblages from 6090 globally distributed time series across six taxonomic groups. We found that local-scale increases and decreases in abundance, species richness, and temporal species replacement (turnover) were intensified by as much as 48% after forest loss. Temporal lags in population- and assemblage-level shifts after forest loss extended up to 50 years and increased with species' generation time. Our findings that forest loss catalyzes population and biodiversity change emphasize the complex biotic consequences of land-use change.

Accelerating human impacts are reshaping Earth's ecosystems (*1*). The abundance of species' populations (*2*, *3*) and the richness (*4*–*6*) and composition (*6*) of ecological assemblages at sites around the world are being altered over time in complex ways (*7*–*9*). However, there is currently only a limited quantitative understanding of how global change drivers, such as land-use change, influence the observed heterogeneous local-scale patterns in population abundance and biodiversity (*8*, *10*, *11*). In terrestrial ecosystems, much current knowledge stems from space-for-time approaches (*12*, *13*) and model projections (*14*, *15*) that attribute population and richness declines to different types of land-use change, including reductions in forest cover. Yet space-for-time methods may not accurately represent the effects of global change drivers, because they do not account for ecological lags (*8*, *16*, *17*) and community self-regulation (*18*). Furthermore, ongoing controversy about the diverse impacts of habitat fragmentation on biodiversity (*19*–*21*) could be in part attributable to a lack of observational data from sites encompassing the full spectrum of forest fragmentation. Recent global-scale datasets of past land cover reconstructions (*22*) and contemporary high-resolution remote-sensing observations (*23*, *24*) provide an opportunity to quantify landscape-scale decreases and increases in forested areas around the world (hereafter, forest loss and gain). By integrating forest loss estimates with population and biodiversity observations (*25*, *26*) (Figs. 1 and 2A), our analysis provides insight into the influence of land-use change on local-scale population and biodiversity change around the planet.

In our study, we set out to conduct a global-extent attribution analysis of the influence of forest cover change on population and biodiversity change (Fig. 1). We quantitatively tested specific predictions of how the timing and magnitude of landscape-scale forest loss influence species' populations and ecological assemblages across terrestrial ecosystems around the planet (Figs. 1 and 2 and table S1) (*27*). Land-use change, and particularly forest cover loss, alters habitat and resource availability (*12*, *28*, *29*) and is a global threat for the persistence of terrestrial species (*30*) (Fig. 2 and fig. S12). We thus predicted the greatest impacts on populations and biodiversity when time-series monitoring encompassed the 10-year period that included the largest reduction in forested areas at each site (calculated between the years 850 and 2015, hereafter "all-time peak forest loss"). We also expected greater population and species richness declines and higher turnover after, relative to before, contemporary peak forest loss (i.e., the year of the largest reduction in forested area within the duration of each time series). Finally, species with longer generation times typically respond more slowly to environmental change (*31*). We thus predicted that lags in ecological responses to forest loss would increase with longer generation times across taxa.

We measured landscape-scale historic and contemporary forest loss by integrating information from the Land Use Harmonization (*22*) and Global Forest Change (*23*) databases. We also used the ESA Landcover (*32*) and KK09 (*33*) databases to examine whether our results were consistent across land-use change data sources. We compared historic and contemporary forest loss with temporal population change (trends in the numerical abundance of species) and biodiversity change (trends in species richness and turnover in assemblage composition) (Figs. 1 and 2). We analyzed 2729 populations of 730 species and biodiversity change in 3361 ecological assemblages (Figs. 2A and 3). We measured population change using the Living Planet Database, which includes 133,092 records of the number of individuals of a species in a given area over time (*25*). We measured biodiversity change using the BioTIME database, which comprises 4,970,128 records of the number and abundance of species in ecological assemblages over time (*26*). Together, these time series represent a range of taxa including amphibians (388), birds (5090), mammals (266), reptiles (76), invertebrates (80), and plants (187) and 2157 sites that cover almost the entire spectrum of forest loss and gain around the world (Fig. 2B). We used a standardized cell size of approximately 96 km^2 to match response variables (population change, richness change, and turnover) to landscape-scale forest change. Our analyses were robust to the spatial scale over which we calculated forest change (figs. S13 and S14) (*27*).

We carried out the following workflow for our global assessment of the consequences of forest cover change for population and biodiversity trends over time. To relate population and biodiversity change to historic forest loss, we quantified the baseline all-time peak forest loss at each site. To relate population and biodiversity change to contemporary forest loss, we compared population and biodiversity change before and after contemporary peak forest loss. To investigate temporal lags, we quantified the time period between contemporary peak forest loss and maximum change in populations and assemblages detected after peak forest loss had occurred at each site (Fig. 1B). We calculated population change (μ) using state-space models that account for observation error and random fluctuations (*34*), and calculated richness change (slopes of rate of change over time) using mixed-effects models. We quantified temporal change in species composition as the turnover component of Jaccard's dissimilarity measure (change due to species replacement) (*35*). Turnover is often independent of changes in species richness (*9*) and is the dominant component of compositional change across time series of ecological assemblages (*36*). We used a hierarchical Bayesian modeling framework, with individual time series nested within biomes (*37*), to account for the spatial structure of the data (*27*).

Historical baselines

In line with our first prediction ("historical baselines"), we found that local-scale population declines were more pronounced when the monitoring occurred during the period

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of all-time peak forest loss (Fig. 1B and Fig. 3, B and C). For many of the sites represented by the time series we studied, major changes in forest cover occurred during the past two centuries. In regions such as Europe and North America, all-time peak forest loss was typically during the early 1800s, before population, biodiversity, and satellite monitoring had begun (Figs. 2C and 3B). The Living Planet Database and BioTIME time series captured more than half of the spectrum of contemporary forest cover change around the world, in contrast to previous criticisms of some of these data underrepresenting areas with anthropogenic impact (*38*) (Fig. 2, B and C, and Fig. 3B). Yet in only ~5% of monitored time series did forest loss lead to a conversion in the dominant habitat type (e.g., from primary forest to urban areas). Habitat conversions corresponded with both gains and losses in populations and biodiversity, with the highest rates of turnover when primary forests were converted to agricultural and urban areas or to secondary forests (fig. S17). The links among historical baselines, the timing of all-time peak forest loss, and resulting ecological change emphasize the need for a long-term perspective to quantify the complexity of biodiversity change in the Anthropocene (*11*, *17*).

Contemporary forest loss

Contrary to our second prediction ("contemporary forest loss"), we found that forest loss acted as a catalyst amplifying both increases and decreases in local-scale populations and assemblages over time (Figs. 3 and 4 and figs. S4 to S6, S9, and S10). Across time series, more than half of all populations and assemblages (61%) experienced higher rates of change after the largest forest loss event within each time series. Contemporary peak forest loss intensified population declines, population increases, and richness losses—but not richness gains—relative to the period before peak forest loss (Fig. 4). In nearly one-third of time series (32%), more than 10% of the species in the assemblage at the time of contemporary peak forest loss were replaced by new species by the end of the time series (Fig. 4, G and H). The assemblages that experienced the most richness change also experienced the most turnover [Pearson correlation = 0.37; 95% confidence interval (CI) = 0.31 to 0.43]. The influence of contemporary peak forest loss on population and biodiversity change was not strongly correlated to the magnitude of the specific forest loss event (figs. S4 to S6). Our findings indicate a wide spectrum of population and biodiversity responses to forest loss that might be overlooked without accounting for temporal dynamics and lagged responses (*12*, *13*, *15*, *39*).

Temporal lags

In line with our third prediction ("temporal lags"), we found evidence for up to half-century ecological lags in local-scale changes in population abundance, species richness, and turnover after contemporary peak forest loss (Fig. 5). On average, we documented maximum change in populations and ecological assemblages 6 to 13 years after forest loss across taxa. Yet nearly half of population and biodiversity change (40%) happened within 3 years of peak forest loss, demonstrating that rapid shifts in populations and assemblages occur frequently after habitat change (Fig. 5 and fig. S7). Consistent with our prediction, the

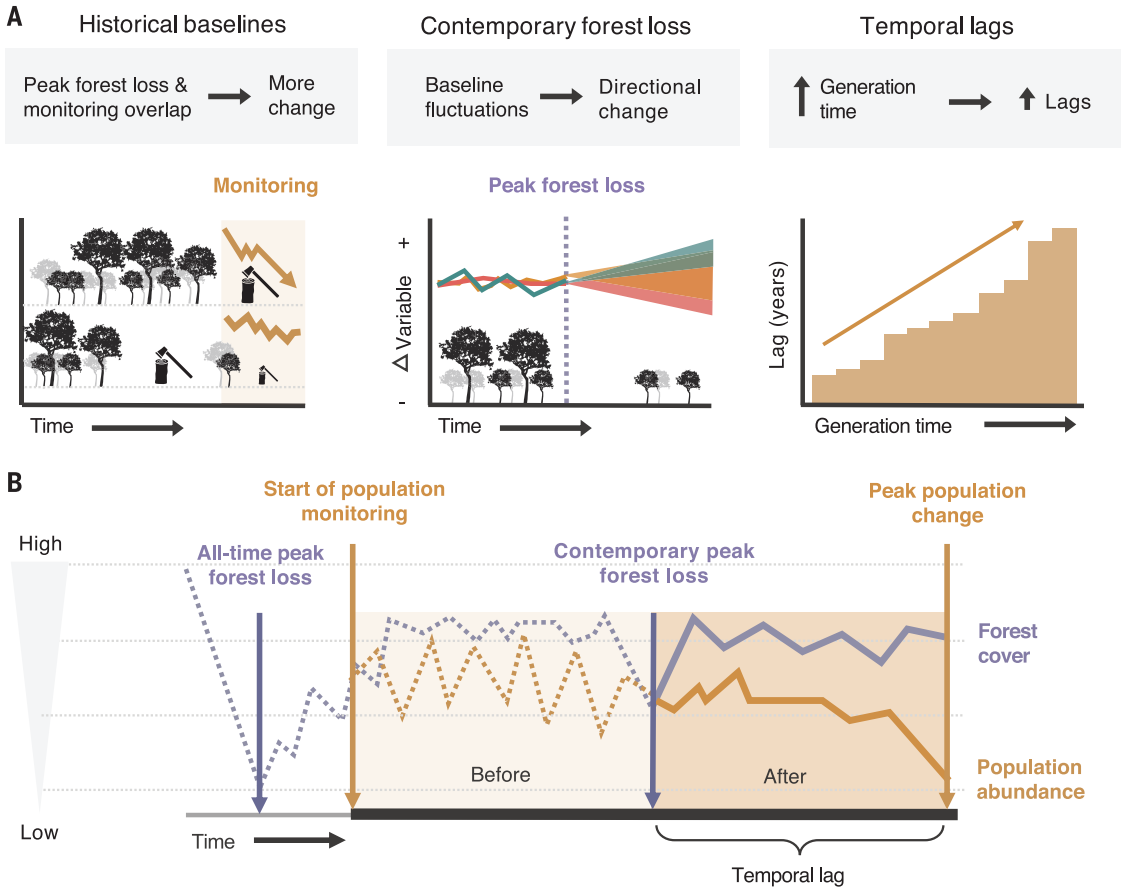

Fig. 1. Influence of forest loss on population and biodiversity change. We tested three pathways through which forest loss can influence the population abundance of species and the richness and turnover (temporal species replacement) of ecological assemblages: historical baselines of forest loss, timing of contemporary forest loss, and temporal lags in population and biodiversity responses. (**A**) Conceptual diagram of our predictions outlined with respect to population change, richness change, and turnover. (**B**) Analytical workflow for determining all-time and contemporary peak forest loss and temporal lags [further details in (*27*)].

period between peak forest loss and peak change in populations and biodiversity was longer for taxa with longer generation times (e.g., large mammals and birds; Fig. 5B and table S2). Population declines and increases occurred on similar time scales (Fig. 5C). Losses in species richness lagged behind gains by approximately half a year (slope = 0.5, CI = 0.1 to 1.05), indicating that extinction debts and immigration credits accumulated at roughly the same speed across taxa. The similar pace and temporal delay of population declines and increases, and of richness gains and losses, could help to explain previous findings of community self-regulation (*18*) and no net population

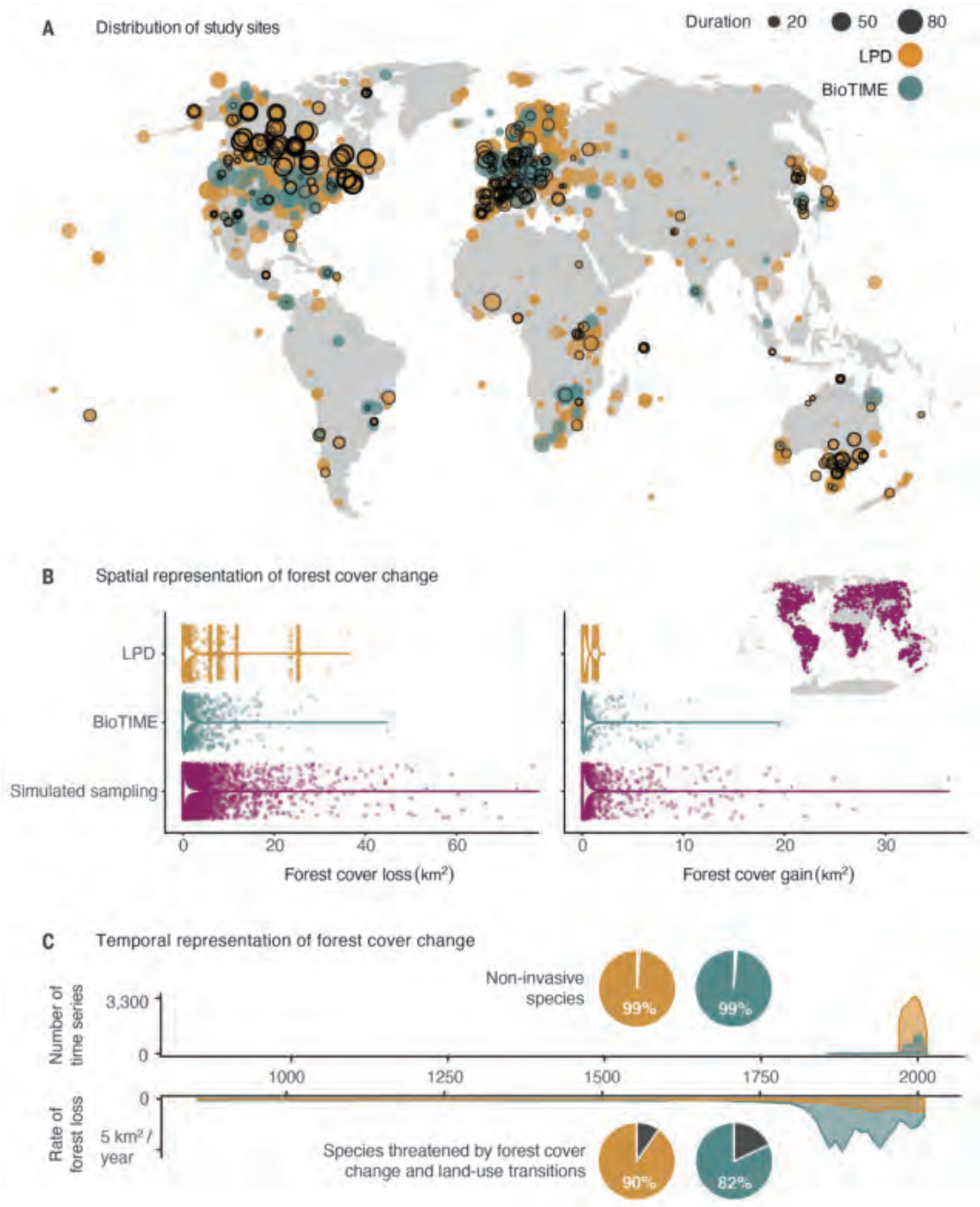

Fig. 2. Population and biodiversity monitoring over time broadly spans the global variation in forest cover change. (**A**) Locations and duration of 542 Living Planet Database (LPD) and 199 BioTIME studies, containing 6090 time series from 2157 sites [black outline denotes sites that were forested at the start of the monitoring (1247 sites); see table S1 for sample size in each woody biome]. (**B**) Among all time series, 44% experienced historic or contemporary forest loss of comparable magnitude to forest cover change across a simulated random sample of geographical locations (shown on map inset) from the global distribution of forest cover loss and gain. We did not detect directional effects of the magnitude of forest gain across monitored sites (figs. S4 to S6). (**C**) The number of time series increased over time (top), but the rates of forest loss were often higher before the start of monitoring (bottom; see figs. S2 and S3 for variation in monitoring periods among time series). Insets show the proportion of study species that are not classified as invasive (top) and that are threatened by land-use change, based on species' IUCN threat assessments (bottom; see fig. S12 for details).

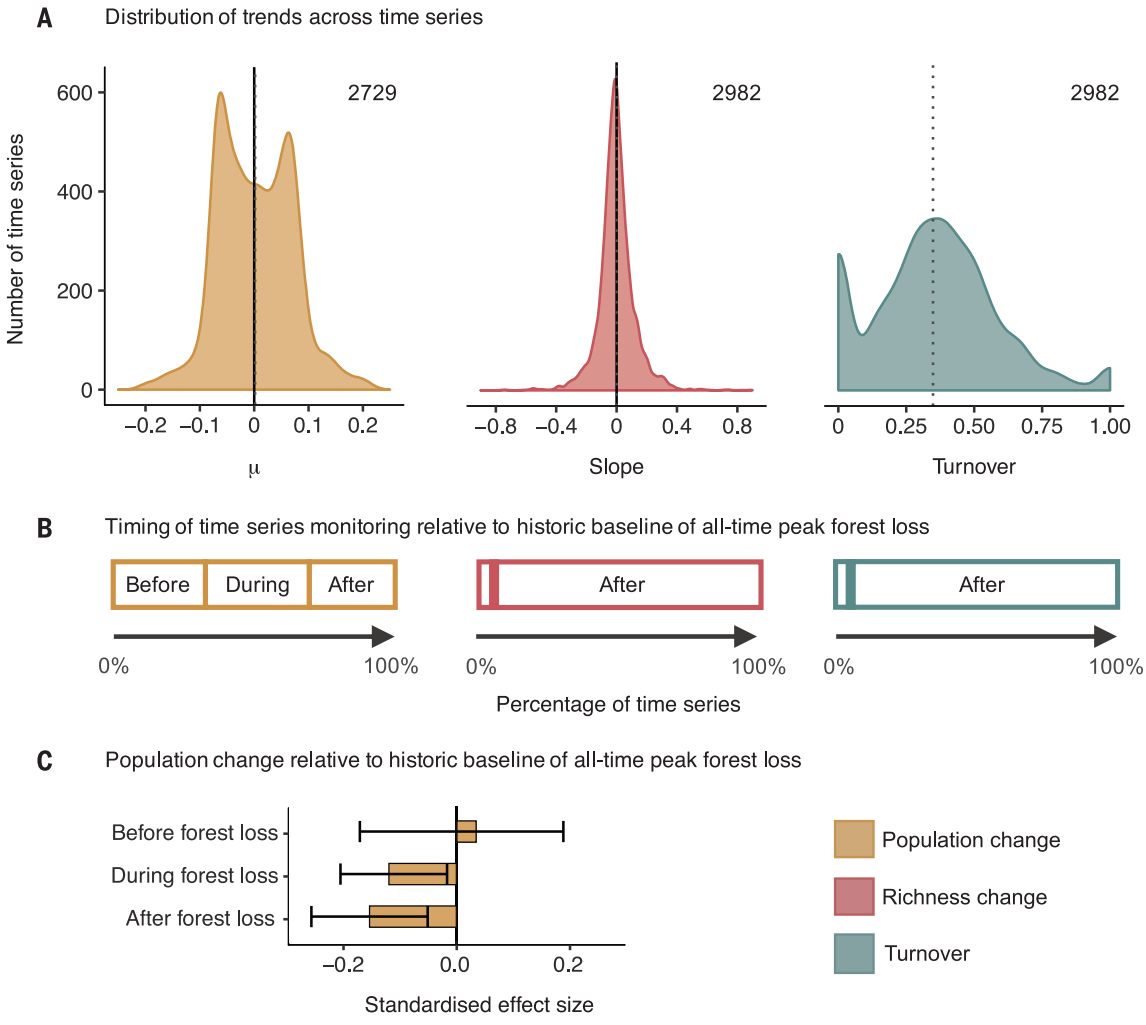

Fig. 3. Heterogeneity in population and biodiversity trends and land-use histories from sites around the world. (**A**) All three metrics of ecological change (population change, richness change, and turnover) show heterogeneous distributions across sites. (**B**) Population monitoring occurred at different time periods relative to all-time peak forest loss (for 33% of sites before, for 37% during, and for 30% after), whereas biodiversity monitoring predominantly started after all-time peak forest loss had occurred (94% of sites). (**C**) Population declines were more acute when all-time peak forest loss occurred during the population monitoring period (slope = −0.007, CI = −0.012 to −0.001; see table S2 for model outputs). Low sample size for the "before" (101) and "during" (38) categories precluded a similar analysis for richness change and turnover. Lines in (A) denote zero (solid) and mean values (dotted). Numbers in (A) show sample size (i.e., number of time series).

change (*2*, *3*, *10*) or richness change (*5*, *6*) at local scales. Temporal lags in biodiversity change have also been observed in post-agricultural forests (*4*, *40*) and fragmented grasslands (*31*), where agricultural activity ceased decades to centuries ago, yet richness and assemblage composition change continue to the modern day. Overall, our results indicate that increasing rates of land-use change in the Anthropocene (*41*, *42*) will alter ecosystems on both short- and long-term time scales that need to be captured in ongoing and future biodiversity monitoring.

Heterogeneity in responses to forest cover loss could be due to a number of factors, including (i) temporal lags in population or assemblage responses, as observed in our study and elsewhere (*17*, *31*); (ii) context-specific responses to forest loss, such as the same amount of habitat change corresponding to biodiversity declines at one site but increases at another (*13*, *43*, *44*); and (iii) interactions with other drivers occurring simultaneously with forest loss (*11*, *45*, *46*). Our finding that forest loss was concurrent with both declines and increases in populations and assemblages is consistent with the varied and often positive effects of habitat fragmentation on biodiversity metrics such as species richness (*19*). However, forest loss occurring outside of the period of population or biodiversity monitoring, as well as the type of woody vegetation being gained and lost, might influence our ability to detect a causal link between forest loss and biodiversity change (*17*, *47*). Increases in woody vegetation caused by agroforestry or plantations might not reflect ecosystem recovery, such as with natural succession after forest cover loss (*48*–*50*). Our finding that forest cover gain did not directly correspond with gains in population abundance and species richness highlights the need for high-resolution temporal data of the specific vegetation types constituting forest cover changes around the world. The complexity and heterogeneity of forest cover change effects on biodiversity (*13*, *43*, *51*, *52*) demonstrate that caution is warranted with respect to recent calls for global afforestation as a climate change mitigation tool (*53*).

Variation in species' vulnerability to forest cover loss (*43*, *51*) may be contributing to the wide spectrum of population and biodiversity responses to shifts in forest cover. Species that have experienced frequent habitat disturbance during their evolutionary history might be more resilient to land-use change, whereas novel habitat alterations could have a greater influence on species' persistence and abundance (*13*, *43*) (Fig. 3). In a post hoc test, we found that in forest-dominated sites, where past disturbances were likely less frequent, declines in species' abundance were more frequent than increases when contemporary forest loss occurred, whereas richness change and turnover did not show directional trends (fig. S16). Additionally, in our study, rare and common species, as defined by their range size, mean population size, and habitat specificity (*54*), responded in similar ways to forest loss (figs. S11 and S12). In contrast to this result, space-for-time comparisons that do not account for temporal dynamics and lagged responses have found that land-use change has a more negative impact on rare species

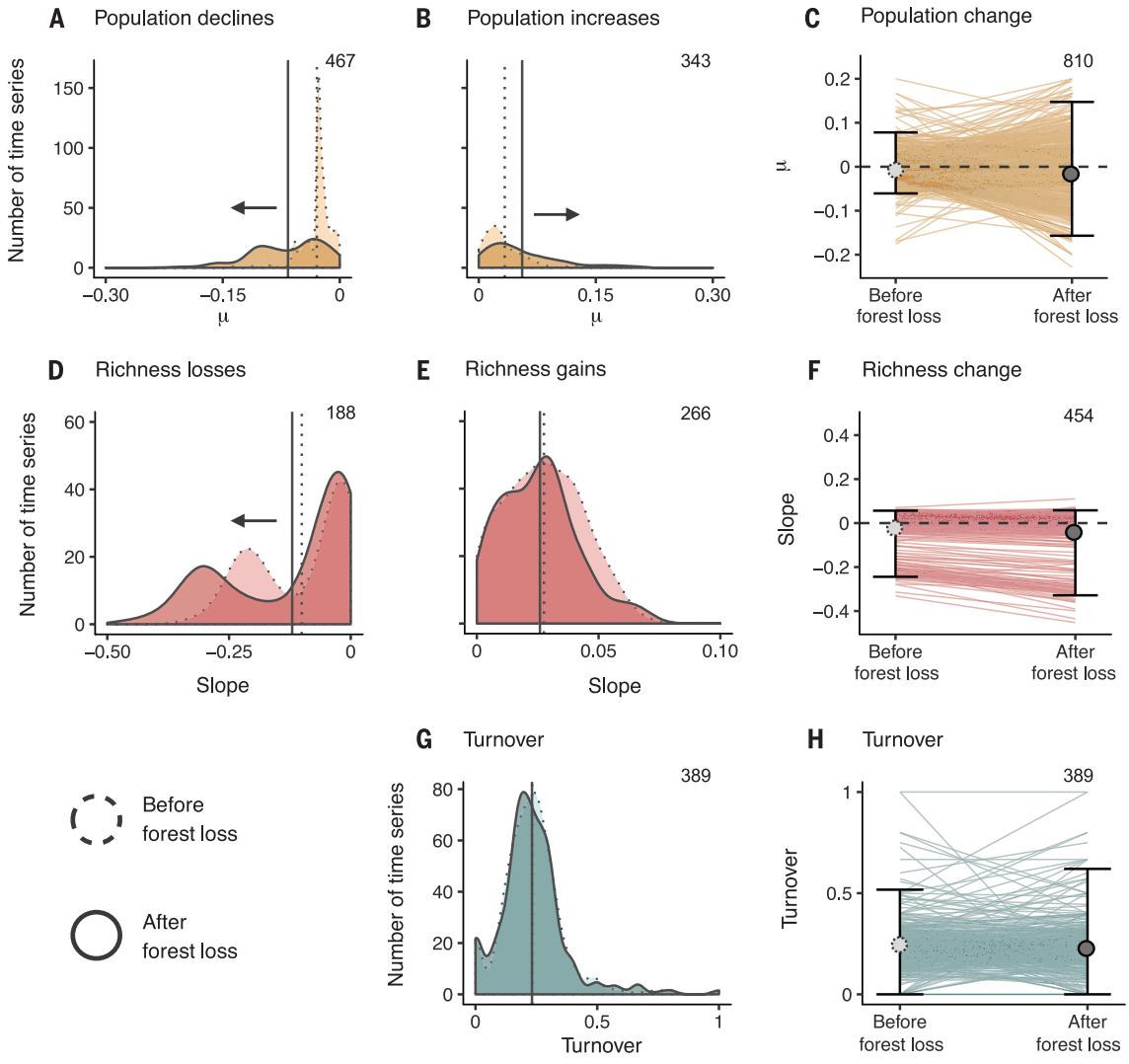

Fig. 4. At the site level, population and biodiversity change increase after contemporary peak forest loss. In total, population and richness change increased across 61% and decreased across 39% of the 1653 time series for which baseline comparisons were possible (i.e., the time series were long enough to include at least 5 years before and after forest loss). Only turnover included instances of no difference in the amount of change before and after peak forest loss (6% of time series). (**A**, **B**, **D**, **E**, and **G**) Distributions compare population declines (μ) (A), population increases (μ) (B), richness losses (slopes) (D), richness gains (slopes) (E), and turnover (Jaccard's dissimilarity) (G) in the periods before and after contemporary peak forest loss, the largest forest loss event during the monitoring of each site. Vertical lines over distributions show the mean for each category (dotted, before; solid, after). (**C**, **F**, and **H**) Temporal trends before and after peak forest loss are indicated with lines for individual time series. Light and dark gray points and error bars show mean values and 2.5% and 97.5% quantiles. Duration varied among time series but was consistent for each individual time series (i.e., *n* years before forest loss = *n* years after forest loss, *n* ≥ 5 years; see fig. S8 for relationship between duration and number of survey points). Numbers on plots indicate sample size. See table S2 for model outputs.

than on common species (*55*). Accounting for both inter- and intraspecific heterogeneity in species' vulnerability to forest cover change is key when scaling from localized impacts of human activities to global-scale biodiversity patterns and attribution of change (*1*, *19*–*21*, *39*, *43*, *51*).

Taxonomic, spatial, and temporal imbalances in sampling can make large-scale attribution analyses of biodiversity trends and global change drivers challenging and can influence the inferences that we draw from such studies (figs. S2, S3, S8, S9, and S11 to S14). For this reason, we explored in greater detail three specific challenges of our terrestrial biodiversity attribution analyses. First, tropical species and locations are underrepresented in current open-source temporal biodiversity databases (Fig. 2A) (*38*). In a post hoc test, we found that in the tropics, where there is intense, often unprecedented forest loss, the effects of forest loss were stronger and more negative across sites with available data, relative to the rest of the globe (figs. S9 and S10 and tables S1 and S2). Second, the spatial scales at which biodiversity is monitored (from 1 m^2 to 25 × 10^8 km^2) and the resolution of forest cover datasets (from 30 m to ~20 km; figs. S13 and S14) could introduce spatial mismatches between the driver and response. Nonetheless, we found that the heterogeneous relationships among richness change, turnover, and forest loss were consistent across forest loss calculated on scales from 10 to 500 km^2 (fig. S14). Third, temporal mismatches and lags (Figs. 1C and 5) can obscure the ways in which forest loss may be related to population and biodiversity change. We found that attribution signals were strongest when a peak in forest loss occurred during the time-series monitoring (Figs. 3 and 4). Our results indicate that biodiversity assessments and global change attribution analyses will be improved by better spatial and temporal matching of biodiversity and environmental impact data.

Our analysis reveals an intensification of both increases and decreases of populations and biodiversity by up to 48% after forest loss at sites around the planet. This finding demonstrates heterogeneity in the influence of forest cover change on populations and ecological assemblages and challenges the assumption that land-use change predominantly leads to population declines and species richness loss (*12*, *14*, *39*). A current assumption underlying existing projections of biodiversity responses to land-use change (*12*, *14*) is that space-for-time approaches accurately reflect longer-term population and biodiversity dynamics (*41*). In contrast, we found temporal

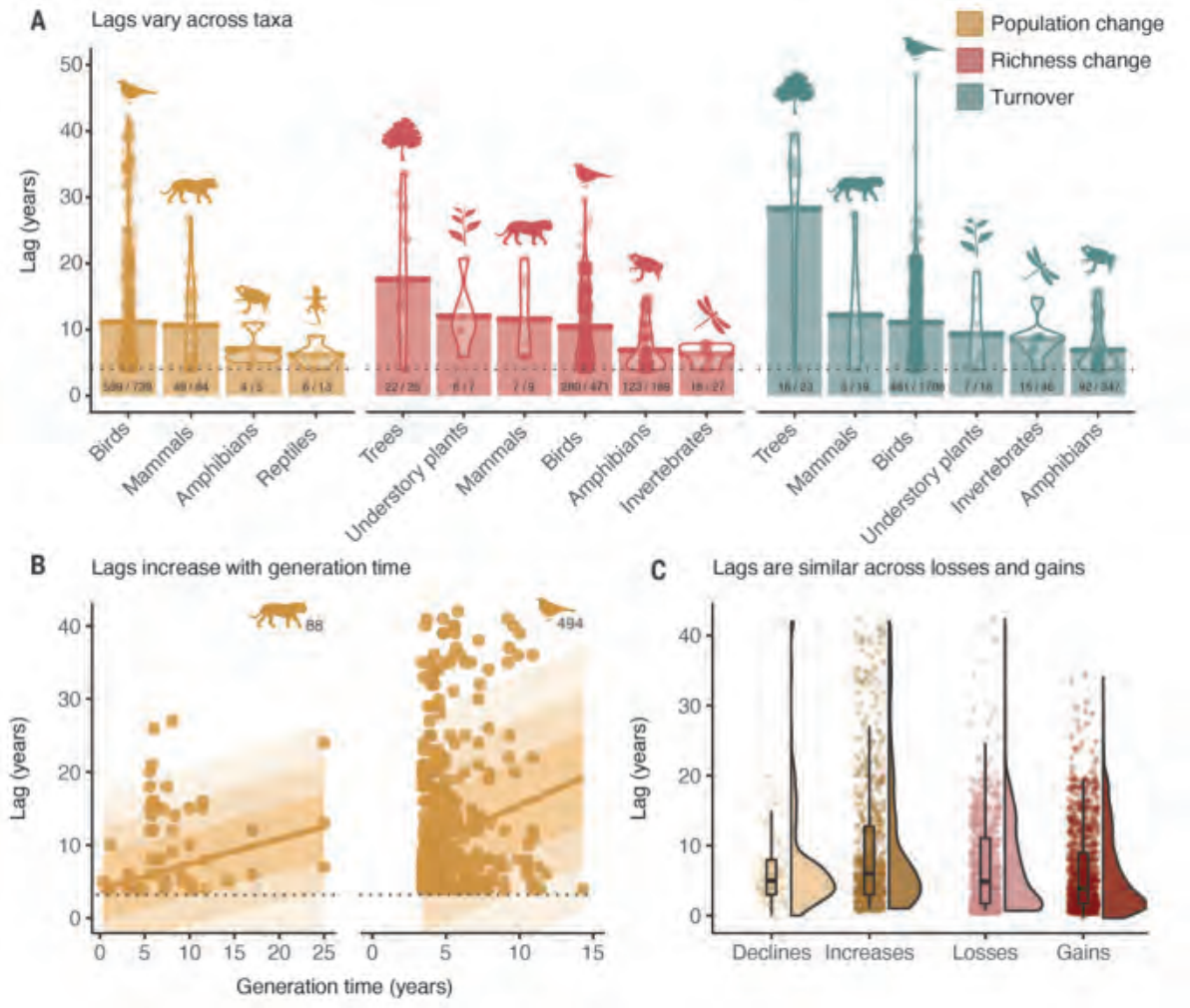

Fig. 5. Temporal lags in population and biodiversity change after contemporary peak forest loss. Population and assemblage change after contemporary peak forest loss may be delayed by up to half a century, with taxa and species with long generation times showing the longest temporal lags. (**A**) We compared lags of 3 (dashed horizontal line) or more years between peak forest loss during the monitoring for each time series and peak change in population or biodiversity across six taxa (sample size was 841 time series for population change, 728 for richness change, and 2157 for turnover). Bars show mean lag for each taxon, violins show the distribution of lag values, and the points are lag values for each time series. Numbers on bars indicate how many time series experienced lags out of the total sample size for each taxon. (**B**) We found that temporal lags in mammal and bird population change increased with longer species generation times. (**C**) Temporal lags were similar across population declines and increases and across species richness losses and gains. See table S2 for model outputs.

lags of up to 50 years in population and biodiversity change after forest loss that differed across taxa and among species' generation times. Our analyses highlight that the local-scale responses of populations and assemblages to forest cover loss and gain are complex and variable over time. Incorporating the full spectrum of population and biodiversity responses to land-use change will improve projections of the future impacts of global change on biodiversity and thus contribute to the conservation of the world's biota during the Anthropocene.

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SUPPLEMENTARY MATERIALS

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REPORTS

METALLURGY

Making ultrastrong steel tough by grain-boundary delamination

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Developing ultrahigh-strength steels that are ductile, fracture resistant, and cost effective would be attractive for a variety of structural applications. We show that improved fracture resistance in a steel with an ultrahigh yield strength of nearly 2 gigapascals can be achieved by activating delamination toughening coupled with transformation-induced plasticity. Delamination toughening associated with intensive but controlled cracking at manganese-enriched prior-austenite grain boundaries normal to the primary fracture surface dramatically improves the overall fracture resistance. As a result, fracture under plane-strain conditions is automatically transformed into a series of fracture processes in "parallel" plane-stress conditions through the thickness. The present "high-strength induced multidelamination" strategy offers a different pathway to develop engineering materials with ultrahigh strength and superior toughness at economical materials cost.

Lightweight yet cost-efficient structural materials with superior mechanical properties are continually sought after in engineering applications to meet the demands of sustainable economy development. Unfortunately, attaining high strength is usually at the expense of deteriorating toughness, which invariably is a major concern for safety-critical applications (*1*, *2*). The strengthening mechanisms in structural metals and alloys are built on the fundamental principle of inhibiting, or blocking, dislocation slip through the introduction of various obstacles at different length scales (*3*, *4*). However, abundant dislocations entangled in small imperfections can give rise to the localized stress concentrations that can cause crack initiation, which eventually can lead to catastropic failure (*1*, *5*). A mainstream effort to overcome the strength-toughness trade-off is focused on tailoring the microstructure or designing materials by means of solid-solution alloying. Multielement high- and medium-entropy alloys possess exceptional damage tolerance at cryogenic temperatures because of a deformation-induced nano-twinning mechanism (*6*, *7*). Maraging steels are another example and are recognized as the strongest metal alloys with acceptable damage tolerance for aerospace applications. However, maraging steels contain a large amount of costly alloying elements such as nickel (17 to 19 wt %), cobalt (8 to 12 wt %), and molybdenum (3 to 5 wt %) (*8*). Although the maraging steel alloying strategy is a perfect vehicle to attain superior mechanical performance, economical mass production and recycling are not feasible because of costs and environmental concerns (*3*, *9*). Reducing the average grain size is a different nonalloying pathway to enhance the strength-toughness combination. However, this approach can be limiting because the strengthening is usually achieved at the sacrifice of losing ductility (*10*, *11*). We show that exceptional damage tolerance can be achieved in an ultrastrong steel, with a simple composition and cost-effective processing route for fabrication. We demonstrate that increasing the yield strength is not detrimental to the toughness but instead can facilitate the activation of a delamination toughening (*12*, *13*) mechanism. This substantially enhances the toughness. Specifically, the ultrahigh-yield strength enables a secondary fracture mode, delamination cracking, at interfaces normal to the primary fracture surface. Multiple separated laminated ligaments develop near the fracture surface because of the delamination events, providing

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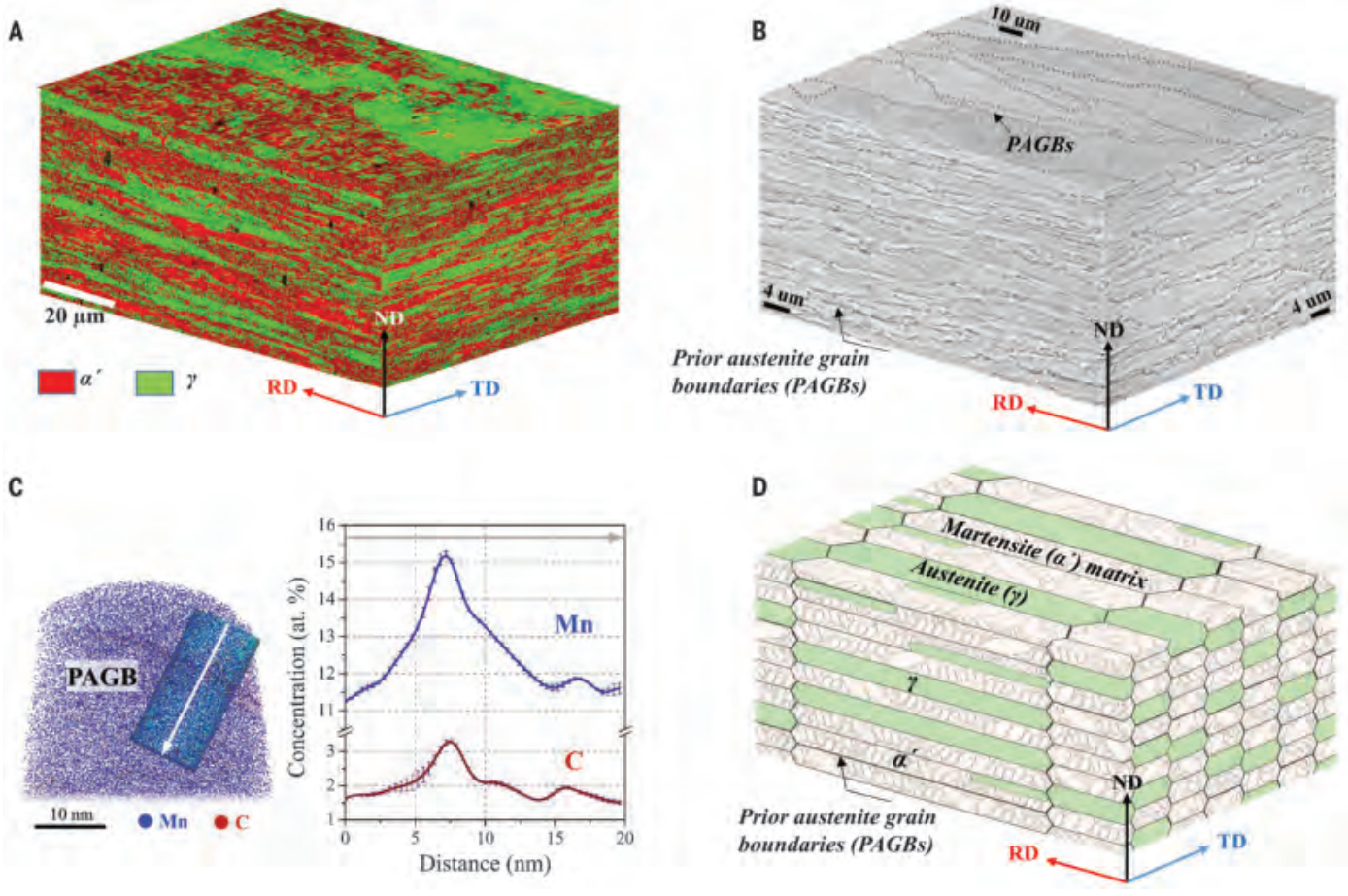

Fig. 1. Microstructure of the present steel. (**A**) 3D stereographic microstructure reconstructed with electron backscatter diffraction (EBSD) phase maps scanned on the orthotropic planes perpendicular to the RD, TD, and ND, respectively. A heterogeneous laminated duplex microstructure comprising martensitic matrix (α′) and elongated austenite (γ) lamellae is exhibited in the present steel. Retained austenite grains are elongated along the RD and slightly stretched along the TD. (**B**) 3D stereographic microstructure reconstructed by using the PAGBs maps imaged with SEM. The PAGBs are indicated by the black dotted lines and show a heterogeneous distribution. (**C**) 3D ion concentration map, which contains a PAGB and a corresponding 1D concentration profile across the boundary and shows the segregation of Mn and C to the PAGB. (**D**) Schematic 3D model illustrates the microstructural features of the heterogeneous microstructure of the present steel.

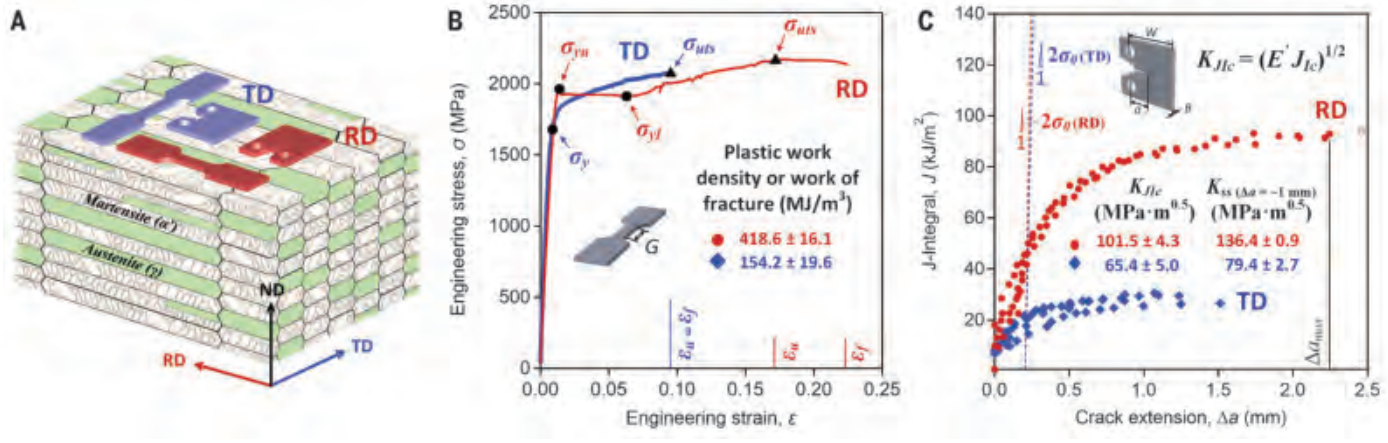

Fig. 2. Tensile and fracture properties of the present steel. (**A**) Schematic diagram describing the orientations of the dog bone–shaped tensile specimens and the C(T) specimens relative to the thin-sheet steel. (**B**) Engineering stress-strain curves of the present steel deformed under tension along the RD and TD orientations. (**C**) The *J*-integral–based resistance curves (*J*-R curves) measured from the C(T) specimens at room temperature.

an extra energy release rate for fracture as well as enhancing crack-tip blunting, collectively increasing the overall fracture toughness. Such delamination toughening combined with transformation-induced plasticity (TRIP) toughening are rarely realized simultaneously in structural materals. The combination enables an intriguing combination of strengh, ductility, and toughness properties in our steel.

Our steel has a chemical composition of Fe-9.95% Mn-0.44% C-1.87% Al-0.67% V (weight percent) and was fabricated by means of a deformed and partitioned treatment (fig. S1) (*14*). Starting with an almost fully austenitic microstructure (fig. S2), the prior-austenite grains were extensively elongated along the rolling direction (RD) during the initial hot rolling and warm rolling processes (*14*, *15*). The austenite partially transforms to martensite during the subsequent cold rolling, resulting in a lamellar martensite-austenite duplex microstructure (Fig. 1A), which contains a volume fraction of austenite, measured with neutron diffraction at 47.5% (fig. S3). Last, partitioning was performed to optmize the mechanical stability of retained austenite by means of C partitioning from martensite to austenite (fig. S4) (*14*). The martensitic matrix (α') is composed of nano-sized grains decorated with intensive dislocations (fig. S5). The dislocation density of martensite matrix was determined by means of neutron diffraction to be 2.43×10^{16} m^{-2}, which is at least one

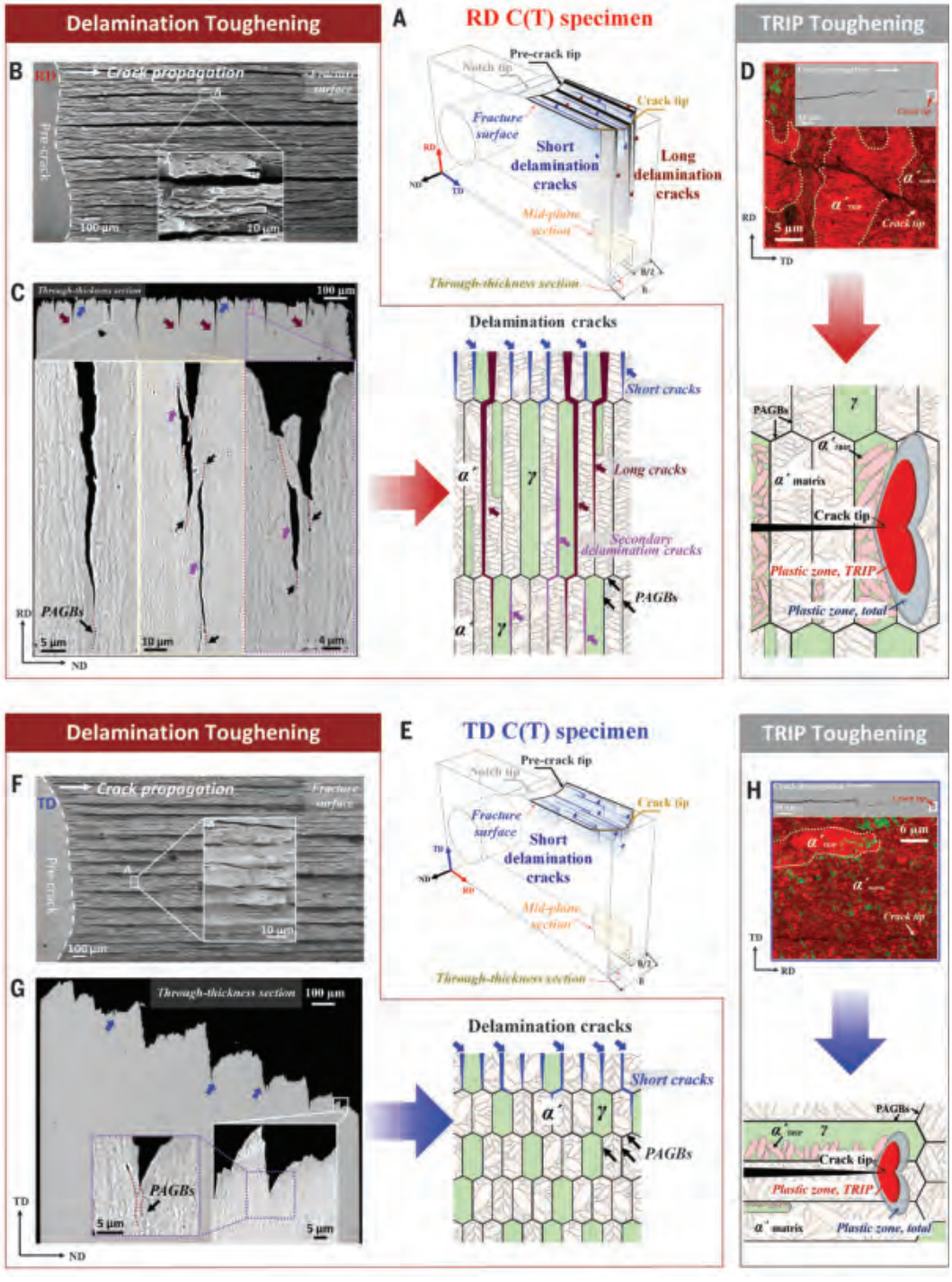

Fig. 3. Toughening mechanisms in the steel. (**A** and **E**) The schematic diagrams of the RD and TD C(T) specimens, respectively, showing the various sections for microstructure characterizations. (**B** and **F**) The fracture surfaces of the RD and TD C(T) specimens, clearly showing the thin-layer delamination bands on the fracture surface. (**C** and **G**) The SEM images captured on the through-thickness section normal to the fracture surface show the development of delamination cracks along PAGBs. Short delamination cracks (blue arrows) and deeply penetrated ones (dark red arrows) are found in the RD specimen, whereas only shallow-penetrated delamination cracks formed in the TD specimen. Slender cracks disconnected from the fracture surface (purple arrows) were usually observed in the vicinity of the long delamination cracks. Corresponding schematic diagrams reveal that the substantially elongated PAGBs in the RD specimen facilitate the propagation of delamination cracks, whereas the short PAGBs and numerous PAGBs perpendicular to the crack propagation in the TD specimen hinder the extension of delamination cracks. (**D** and **H**) EBSD phase maps overlaid with the image quality maps scanned in the vicinity of the main crack tip on the mid-plane section of C(T) specimens. Schematic diagrams delineating the TRIP-toughening mechanism are shown below. The TRIP-induced martensite (α'_{TRIP}) can be distinguished from the martensite matrix (α'_{matrix}) through higher image quality because the α'_{TRIP} grains transformed from the retained austenite have a lower dislocation density (regions bounded by dashed white lines).

order higher than that of other martensitic steels (fig. S6); further details of this calculation based on neutron diffraction measurements are given in (*16*). The austenite (γ) phase displays a stretched lamellar-shaped grain structure with dimensions of hundreds of micrometers along the RD, dozens of micrometers in the transverse direction (TD), and a few micrometers in the normal direction (ND) (Fig. 1A). The prior-austenite grain boundaries (PAGBs) are retained during cold rolling, where some austenite grains transform to martensite; this is indicated with the black dotted lines in the Fig. 1B three-dimensional (3D) stereographic microstructure, which was reconstructed from the PAGB maps. Further atomic-scale composition analysis of the present steel by means of 3D atom probe topography (APT) shows segregation of Mn and C to the PAGB (Fig. 1C) (*16*). No segregation of P, S, or other harmful elements to the PAGB was detected. To better illustrate the laminated duplex microstructure, we constructed a schematic 3D model (Fig. 1D).

To evaluate the mechanical properties of the ultrastrong steel, we characterized the tensile properties and the *J*-integral–based crack-resistance (*J*-Δ*a*) R-curves in both the RD and TD orientations in ambient air. The RD and TD dog-bone–shaped tensile specimens were strained along and perpendicular to, respectively, the elongated austenitic grains (Fig. 2A). Correspondingly, the notch and crack propagation directions in the RD and TD compact-tension [C(T)] fracture toughness specimens were aligned perpendicular and parallel, respectively, to the elongated austenitic grains. Benefiting from the unusually high dislocation density, tensile loading along the RD yielded a superior combination of strength and ductility (Fig. 2B) (*14*). Specifically, the upper yield strength (σ_{yu}), the ultimate tensile strength (σ_{uts}), and the uniform elongation (ε_u) were determined to be 1978 MPa, 2144 MPa, and 19.0%, respectively (Fig. 2B and table S1). Properties in the loading direction along the TD are also sound, with a very high ultimate tensile strength of 2048 MPa, which is similar to that for the RD orientation, but plastic deformation begins to proceed earlier; the 0.2%-offset yield strength (σ_y) in the TD orientation was measured to be 1714 MPa.

In view of its ultrahigh strength and plastic deformation capacity, we evaluated the fracture resistance of the present steel by measuring *J*-integral–based R-curves—*J* as a function of the stable crack extension, Δ*a*—using the C(T) specimens in accordance with the ASTM Standard E1820 (*17*). Our steel displays a modest fracture toughness when loaded along the TD, presenting an average crack-initiation toughness of J_{Ic} = 19.6 kJ·m^{-2} (Fig. 2C and table S1). The crack-resistance (R-curve) behavior in the TD slightly rises as the crack extends, resulting in a valid crack-growth toughness of J_{ss} = 28.7 kJ·m^{-2} at a crack extension Δ*a* of ~1 mm. These fracture properties are comparable with those of the best strong-yet-tough materials, such as 18Ni 300-grade maraging steels (*18*, *19*) and commercial aircraft–quality 300M and 4340 steels (*20*, *21*). In spite of a higher yield strength of the RD tensile specimens, the R-curve in the RD orientation reveals an even better crack resistance, showing a J_{Ic} of 46.9 kJ·m^{-2} and a crack-growth toughness J_{ss} of 84.6 kJ·m^{-2} as the crack extends to Δ*a* ~ 1 mm (Fig. 2C and table S1). These toughness values in the RD are almost 1.5 and 2 times higher than those in the TD, respectively. According to the standard mode-I *J*-*K* equivalence relationship, the crack-initiation toughness (K_{JIc}) of the RD and TD specimens were determined to be 101.5 and 65.4 MPa·m^{½}, respectively. Likewise, the crack-growth toughness at Δ*a* ~1 mm, K_{ss}, was 136.4 and 79.4 MPa·m^{½} for the RD and TD specimens, respectively. These are very high values of the crack-initiation and crack-growth toughnesses in our ultrastrong steel that are not found in any other existing structural materials at a comparable yield strength (~2 GPa).

To illuminate the underlying toughening mechanisms responsible for the exceptional damage tolerance of the ultrastrong steel, we characterized the microstructures on various sections of the RD and TD C(T) specimens (Fig. 3, A and E). Featured regions on the fracture surfaces characterized with scanning electron microscopy (SEM) show the existence of multiple thin-layer delamination bands in both RD and TD orientations (Fig. 3, B and F). Observed from the fracture surface, the original ~1.4-mm-thick RD specimens were delaminated through the thickness into several thin layers with their interspacings (or delamination cracks) "penetrating" into the material along the planes perpendicular to the fracture surface. Moreover, delamination cracks at different length scales were developed in the present steel, resulting in numerous delamination bands sized in the range of several micrometers (Fig. 3B, region A). The thickness of these delamination ligaments was substantially thinner than that in other structural materials that contain delamination cracks (*12*, *13*). Delamination was also present in the TD specimens, but the delamination cracks were fewer in number and shorter in length than those in the RD specimens. The microstructure in the vicinity of the delamination cracks in the through-thickness sections normal to the fracture surface was further characterized to clarify the micromechanisms associated with the delamination cracks (Fig. 3, C and G). On the basis of the statistical distribution of the crack lengths (figs. S7 and S8), the delamination cracks in the RD specimens can be categorized into two groups: short cracks with lengths <~50 μm (Fig. 3C, blue arrows) and longer cracks with lengths >~50 μm (Fig. 3C, dark red arrows). Moreover, slender cracks disconnected from the fracture surface (Fig. 3C, purple arrows) were usually observed in the vicinity of the

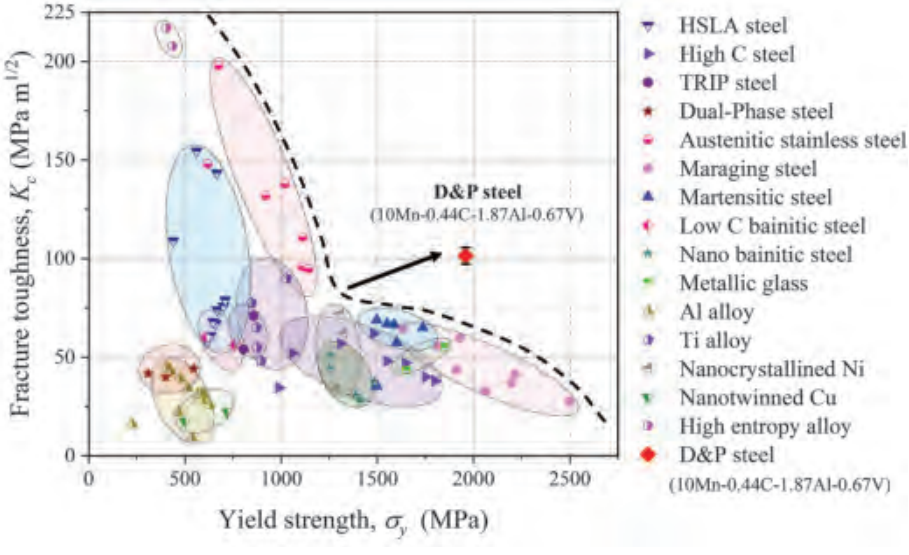

Fig. 4. Ashby map in terms of the fracture toughness versus the yield strength. Our ultrastrong steel overcomes the strength-toughness trade-off shown in most existing structural materials, especially high-strength low-alloy (HSLA) steels (*29*, *30*), high carbon (C) steels (*31*), TRIP steels (*32*), dual-phase steels (*33*), austenitic stainless steels (*34*, *35*), maraging steels (*18*, *19*, *36*), martensitic steels (*37*, *38*), low C bainitic steels (*39*), nano bainitic steels (*40*), metallic glass (*41*), Al alloys (*42*), Ti alloys (*43*), nanocrystallined Ni (*44*), nanotwinned Cu (*45*), and high-entropy alloys (*6*, *7*). Detailed composition and properties of these compared materials can be found in table S2.

long delamination cracks. Further characterization at the tips of the delamination cracks clearly indicated that these cracks propagate predominantly along the PAGBs (Fig. 3C). In the case of the TD specimens, only short delamination cracks with lengths shorter than 50 μm were observed (Fig. 3G). Clearly, loading along the RD results in a much larger tendency for delamination.

The activation of delamination toughening requires two necessary conditions: intrinsically and microstructurally, the existence of "relatively weak interfaces," where delamination takes place; and mechanically, with a "high mechanical stress" that exceeds the critical fracture stress of the "relatively weak interfaces." From our characterization of the locations at delamination crack tips, the Mn-enriched PAGBs in our steel serve as the relatively weak interfaces and preferential sites for the initiation and propagation of the delamination microcracks (Fig. 3, C and G). Actually, the Mn-enriched PAGBs still have a high level of cohesion strength but are just not as strong as the grain interior owing to the segregation of Mn (Fig. 1C) (*22*, *23*). By comparison, such delaminations do not occur in maraging steels, no matter how high their yield strengths are, because there are no such interfaces and preferential sites along the boundaries with reduced cohesion.

From the perspective of the mechanical driving force, a triaxial tensile stress-state exists ahead of a crack tip under plane-strain conditions. The material in front of the crack tip is subjected to a tensile stress (σ_2) (fig. S9A) along the thickness direction (Fig. 1A, ND). When the tensile stress σ_2 that is perpendicular to the PAGBs is sufficiently high to reach the critical fracture stress of the Mn-enriched PAGBs (Fig. 3A), delamination will occur. To meet this requirement, the material should have sufficiently high yield strength so that a high external applied stress is required. A higher external applied stress will lead to a higher σ_2 at the crack tip. For certain structural materials that contain weak interfaces, delamination cracks are mainly formed at low temperatures at which the interfaces may be more brittle and the through-thickness stresses are larger. This leads to the fascinating effect that such alloys—for example, certain Al–Li alloys (*12*) and ultrafine-grained low alloy steels (*24*, *25*)—become tougher at cryogenic temperatures compared with ambient temperature. As demonstrated here, the present steel possesses ultrahigh yield strength because of its high dislocation density and Mn-enriched PAGBs—with relatively low cohesion as compared with the grain interior—acting as the preferential delamination sites; this meets the two necessary requirements to activate delamination toughening and leads to its exceptional room-temperature fracture toughness for a metallic alloy with a yield strength in the 2 GPa range.

As a consequence of the multiple delamination cracks that occur ahead of the crack front and perpendicular to the crack plane, the expected fracture under plane-strain conditions is transformed into a series of fracture processes in "parallel" plane-stress ligaments through the thickness that individually display a far higher (plane-stress) toughness than for a single (full-thickness) plane-strain section (fig. S9B). In parallel, numerous new interfaces generated during the delamination process consume energy, which effectively increases the energy release rate to contribute to the exceptional fracture toughness. Our current study further reveals that the delamination toughening is affected by the relative orientations of the delamination crack path with respect to the elongated duplex structure. The lengths of the PAGBs along the RD are almost three times the lengths of the PAGBs parallel to the TD because of the large rolling reduction (Fig. 1B). As the delamination cracks propagate along the elongated PAGBs parallel to RD in the RD specimens, longer delamination cracks are developed (Fig. 3C). However, delamination cracks extend with short PAGBs parallel to the TD on the through-thickness section in the TD specimen. Furthermore, numerous PAGBs, aligned perpendicular to the crack path, are developed in the TD specimen. These grain boundaries are effective obstacles to retard the propagation of delamination cracks (Fig. 3G). Therefore, the longer delamination cracks developed in the RD specimens and the shorter delamination cracks developed in the TD specimens give rise to a larger and smaller toughening effect, respectively, which is consistent with the higher and lower respective values of the measured toughness in these orientations.

The present steel also displays a TRIP effect during crack advance. The TRIP-toughening is due to an in situ austenite-to-martensite transformation that is triggered in the vicinity of the crack tip, where an initial duplex martensitic-austenitic microstructure (Fig. 1A) is transformed to an almost fully martensitic microstructure (Fig. 3, D and H) after crack propagation. Because of the lattice-parameter difference between martensite and parent austenite, a compressive residual stress is likely introduced by the TRIP effect in the vicinity of crack tip to further resist crack propagation (*26*). In addition, TRIP effect can serve to promote strain hardening, which in turn enhances the uniform ductility by delaying the onset of the necking instability to provide additional intrinsic toughening to resist crack propagation (*27*, *28*).

Similar to the delamination toughening, the TRIP-toughening is also a function of the crack extension orientations. A larger-sized TRIP zone is realized in the RD specimen (Fig. 3D), where the main crack propagates perpendicular to the elongated austenite. By contrast, a small-sized TRIP zone is produced (Fig. 3H) in the TD specimen because the growth of the TRIP zone is substantially restricted by the PAGBs parallel to the elongated austenite lamellae (*28*). This is illustrated in the schematic figures in Fig. 3, D and H. Consistently, higher toughness values exhibited in the RD specimens are ascribed to the stronger TRIP toughening effect induced by the larger-sized TRIP zone, and lower toughness values exhibited in the TD specimens are ascribed to the weaker TRIP toughening effect induced by the smaller-sized TRIP zone.

To demonstrate how the damage tolerance in our steel compares with other ultrahigh-strength structural materials, we show in Fig. 4 an Ashby map of the crack-initiation fracture toughness versus yield strength. Our steel exhibits a yield strength comparable with some of the strongest existing metallic materials—namely, maraging steels—but with an initiation toughness (K_c) that is almost a factor of two greater. Our steel displays a toughness comparable with that of titanium alloys but with a strength that is greater by a factor of two. The combination of high strength and high toughness clearly demonstrates that the "high-strength induced multidelamination" mechanism can be highly effective in maximizing the mechanical properties of high-strength structural materials while minimizing material cost. Our material design principle, which exploits high strength combined with relatively weak interfaces, is one that we believe can be widely applied to optimize the mechanical performance of materials with ultrahigh strength.

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SUPPLEMENTARY MATERIALS

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ELECTROCHEMISTRY

Microfluidic electrochemistry for single-electron transfer redox-neutral reactions

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Electrochemistry offers opportunities to promote single-electron transfer (SET) redox-neutral chemistries similar to those recently discovered using visible-light photocatalysis but without the use of an expensive photocatalyst. Herein, we introduce a microfluidic redox-neutral electrochemistry (μRN-eChem) platform that has broad applicability to SET chemistry, including radical-radical cross-coupling, Minisci-type reactions, and nickel-catalyzed C(sp^2)–O cross-coupling. The cathode and anode simultaneously generate the corresponding reactive intermediates, and selective transformation is facilitated by the rapid molecular diffusion across a microfluidic channel that outpaces the decomposition of the intermediates. μRN-eChem was shown to enable a two-step gram-scale electrosynthesis of a nematic liquid crystal compound, demonstrating its practicality.

Over the past decade, pioneering developments of visible-light photocatalysis in organic synthesis have enabled previously inaccessible redox-neutral reactions that proceed through single-electron transfer (SET) processes (*1*, *2*). Nonetheless, the use of photocatalysts, mostly precious metal complexes (*3*) or sophisticated organic dyes (*4*), could have practical limitations, such as the nontrivial tuning of redox potentials; high cost of transition-metal photocatalysts at scale (*5*); incompatibility of photocatalysts with strong nucleophiles, electrophiles, or radical intermediates (*6*, *7*); and challenging removal of transition metals during purification of the products (*8*, *9*). Electrosynthesis, on the other hand, is an emerging redox platform accessing environmentally benign, cost-effective, scalable, and distinctive transformations (*10*) powered by inexpensive electricity. Consequently, we considered whether electrochemistry could be applied in a practical photocatalyst-free system for SET redox-neutral reactions.

Most of the reported synthetic electrochemistry relies on reactions on a single electrode with by-products generated on the other electrode, and, as such, the nature of the desired electrochemical transformations is either oxidative or reductive (*10*). In contrast, redox-neutral electrochemistry (i.e., paired or coupled electrosynthesis), which involves two desirable half-electrode reactions performed simultaneously, is underdeveloped, despite its relative material and energy efficiency (*10*–*12*), and examples of radical-based redox-neutral electrosynthesis are even rarer (*13*–*15*). In conventional paired electrochemistry setups, the difficulties associated with matching the generation and interelectrode transport rates of the different highly reactive intermediates pose substantial obstacles to achieving the selective transformation over alternative undesired pathways. Microfluidics, however, has been shown to offer controllable and rapid species transport within a micrometer channel (*16*), with recent applications in electrosynthesis for improved reaction performance (*17*, *18*). To further exploit the capability of microfluidic electrochemistry, we sought to develop a microfluidic redox-neutral electrochemical (μRN-eChem) platform to overcome the challenges of photochemistry-inspired SET redox-neutral reactions that involve reactive intermediates generated from both electrodes.

SET radical-radical cross-coupling reactions were selected as the target transformations, because they offer a strategy to perform C(sp^2)–C(sp^3) bond formation without transition-metal catalysts (*19*). In photocatalytic implementations, a persistent radical and a transient radical are consecutively generated within a photocatalytic cycle in homogeneous solution and subsequently combine to give the selective cross-coupling product (*20*, *21*) (Fig. 1A). In contrast, in the electrochemical system (Fig. 1B), the delocalized generation of the persistent radical (P•) and the transient radical (T•) from two separate electrodes requires P• to migrate to the surface of the other electrode surface in order to react with T•. In conventional electrochemical setups, in which electrode distances typically range from millimeters to centimeters, the selective cross-coupling of P• and T• is hindered by radical decomposition due to the inefficient mixing of the two radicals. In a microfluidic parallel flow channel, the interelectrode radical transport is primarily controlled by molecular diffusion, the characteristic time of which can be

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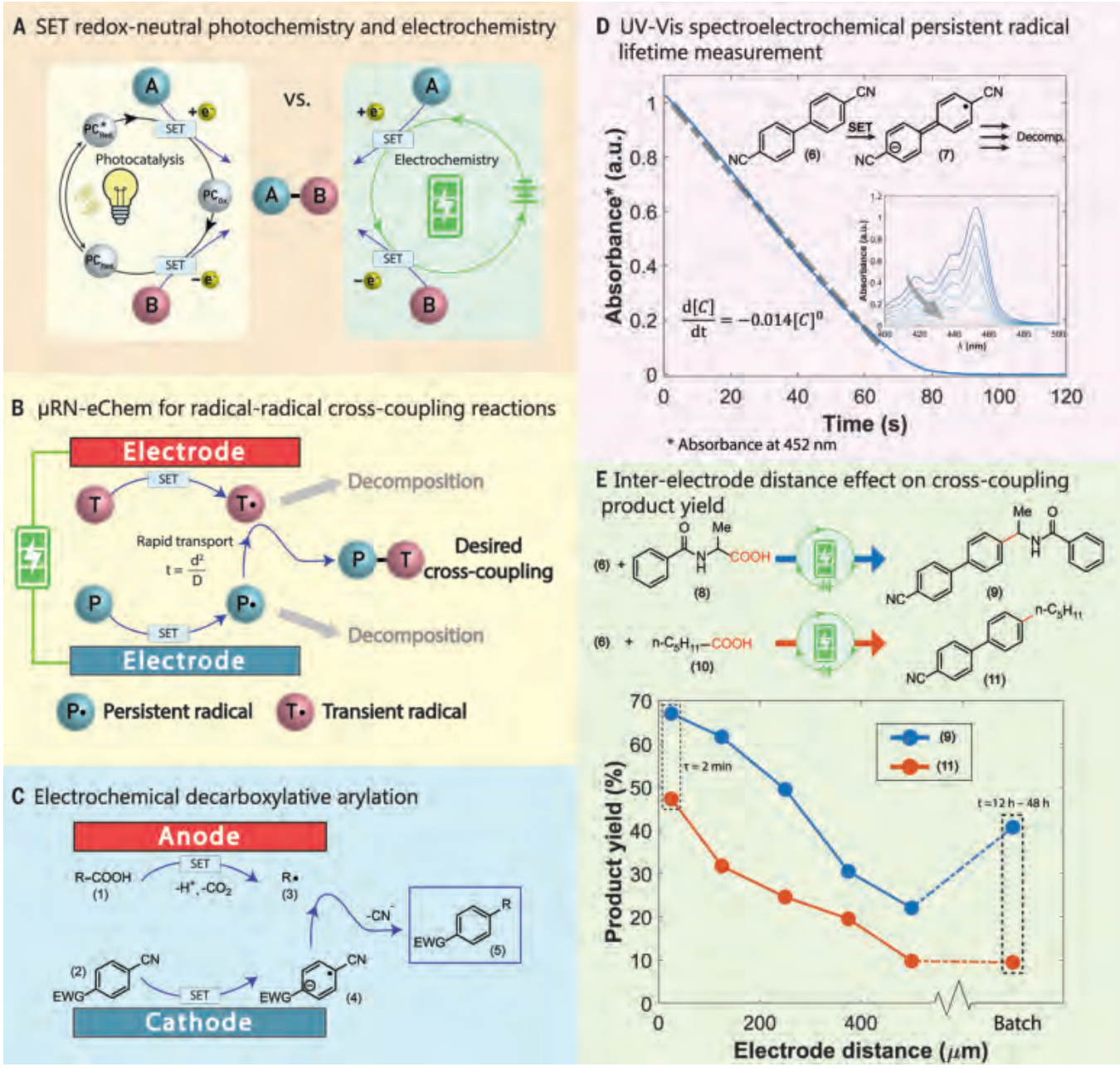

Fig. 1. Background and microfluidic redox-neutral electrochemistry (μRN-eChem). (**A**) Comparison of photochemistry and electrochemistry for SET redox-neutral reactions. PC, photocatalyst; PC_{Red}, ground-state PC; PC^*_{Red}, excited-state PC; PC_{Ox}, oxidized-state PC. (**B**) Concept of μRN-eChem for the cross-coupling reaction of persistent and transient radicals. (**C**) Mechanism of redox-neutral electrochemical cross-coupling reaction of carboxylic acids and electron-deficient aryl nitriles. R–COOH, carboxylic acid (where R is the alkyl group); R•, alkyl radical; EWG, electron-withdrawing group. (**D**) UV-Vis spectroelectrochemical lifetime measurement of BPDN radical anion. a.u., arbitrary units; Decomp., decomposition; [*C*], concentration; λ, wavelength. (**E**) Effect of interelectrode distance on reaction yield. Two batch setups were tested (fig. S5), and the setup with higher yield is presented in this plot. Me, methyl.

estimated by $t = d^2/D$ (*22*), where d is the interelectrode distance, and D is the molecular diffusivity. Hence, we hypothesized that an extremely thin interelectrode gap flow cell would permit rapid diffusion that can outpace the radical decomposition, selectively yielding the radical-radical cross-coupling product.

To test the proposed μRN-eChem platform, we selected model precursors for the transient and persistent radicals (Fig. 1C). Decarboxylative Kolbe electrolysis (*23*), the oldest electrosynthetic reaction (discovered in 1847), exemplifies an anodic alkyl radical generation pathway that is attractive owing to the wide availability of carboxylic acids. For the cathodic persistent radical generation, we selected electron-deficient aryl nitriles (*24*) as precursors. An electron-deficient aryl nitrile **2** can undergo a SET event on the cathode to form a persistent radical anion **4** at a half-wave potential ($E_{1/2}$) of –2.01 V [versus the ferrocene/ferrocenium redox couple (Fc/Fc^+) on glassy carbon (GC) in acetonitrile (MeCN)]. Meanwhile, the deprotonation of alkyl carboxylic acid **1** with a Brønsted base gives an alkyl carboxylate anion, which can undergo SET oxidation at a half-peak potential ($E_{p/2}$) of +0.93 V

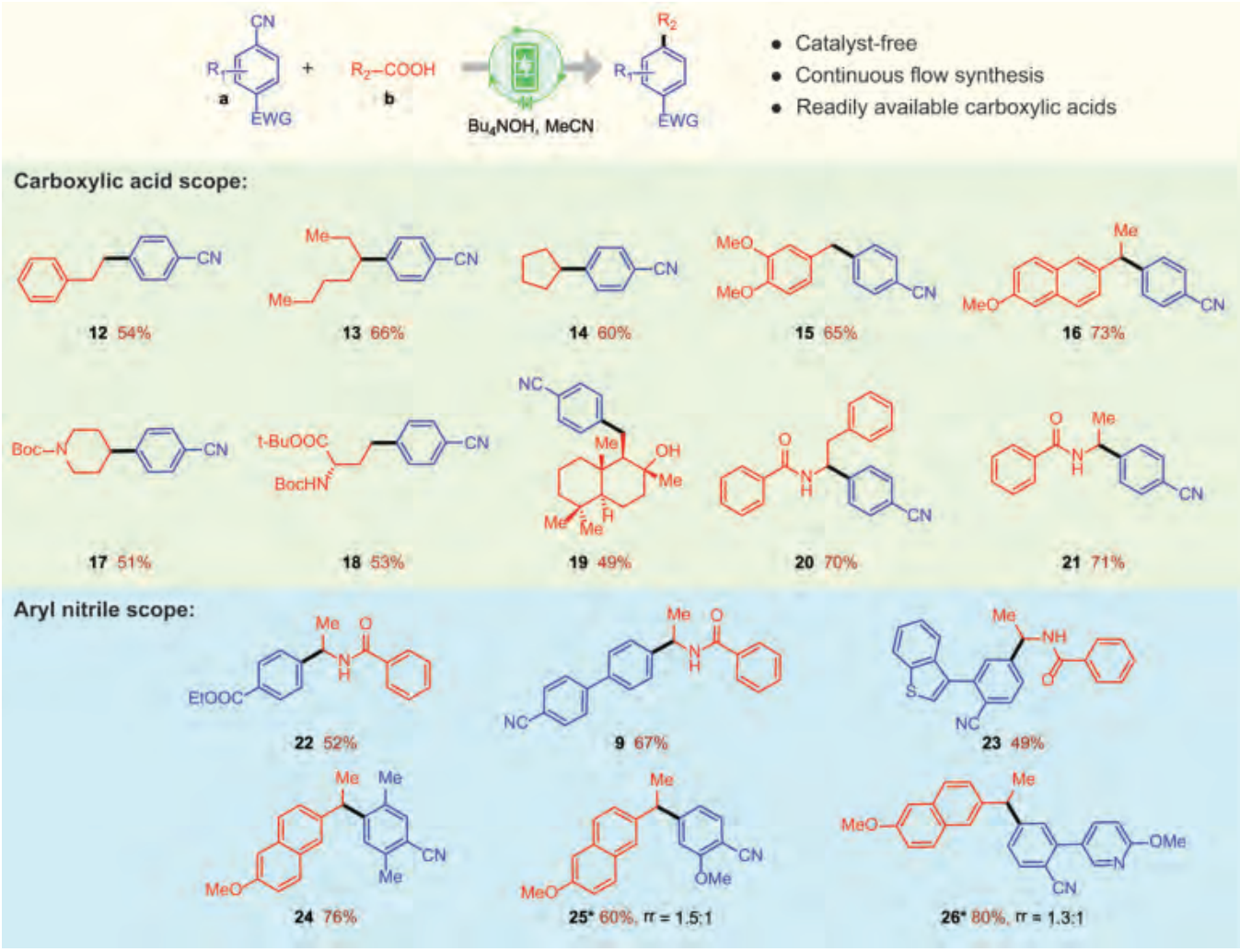

Fig. 2. Substrate scope of decarboxylative arylation in continuous-flow synthesis enabled by μRN-eChem. See the supplementary materials for detailed reaction conditions for each substrate; reactions were performed on a 0.4-mmol scale, unless otherwise noted. Asterisks indicate isomers observed, and in all cases the major isomer is depicted. Yields refer to the combined yield of all isomers. Boc, *tert*-butyloxycarbonyl; t-Bu, *tert*-butyl; Et, ethyl; rr, regioisomeric ratio.

(versus Fc/Fc^+ on GC in MeCN) with subsequent rapid loss of CO_2, yielding a transient alkyl radical **3**. Although such a broad electrochemical window would typically require screening and tuning of photocatalysts to achieve thermodynamically favorable radical generation (*25*), electrochemistry can easily initiate the redox reactions at the proper potential setting.

We engineered a μRN-eChem flow cell with a variable interelectrode distance (25 to 500 μm). Two GC plate electrodes, micromachined by a 532-nm laser, sandwich a thin fluorinated ethylene propylene film to create the microfluidic channel (see fig. S1). 4,4′-Biphenyldicarbonitrile (BPDN, **6**) was selected as a representative persistent radical precursor, and ultraviolet-visible (UV-Vis) spectroelectrochemical measurement of its radical anion **7** showed zero-order decomposition kinetics (Fig. 1D). This radical anion was tested in cross-coupling reactions with two types of transient $C(sp^3)$-centered radicals: a secondary alkyl radical with stabilization by an α-N and a primary alkyl radical without stabilization, generated from corresponding carboxylic acids **8** and **10**, respectively (Fig. 1E). The yields of both cross-coupling products (**9** and **11**) diminished with increasing interelectrode distance. Notably, the yield for cross-coupling of **7** with the α-N stabilized transient radical was less sensitive to interelectrode distance compared with the more reactive primary radical without stabilization. Furthermore, in a batch setup, **9** can still be produced in a reasonable yield (40%) (*15*), whereas only a very low yield of coupling product (9%) was obtained for **11**. The smallest interelectrode distance we examined (25 μm) offers a subsecond molecular diffusion time that is substantially shorter than the lifetime of **7**, leading to a greatly improved cross-coupling selectivity compared with conventional electrochemical setups.

Reaction optimization (see table S1) within the 25-μm flow cell identified Bu_4NOH and MeCN as the optimal base and solvent, respectively. Six equivalents of the carboxylic acids were used to obtain optimal yields; however, a smaller quantity (3 equiv) can be used, albeit with slightly diminished efficiency (9% reduction in yield). No additional supporting electrolyte was required, because of the excellent conductivity in the microfluidic channel. Next, we focused our attention on the scope of carboxylic acids and aryl nitriles that could be successfully transformed (Fig. 2). An extensive range of carboxylic acids with various functional groups proved to be suitable substrates in the cross-coupling with 1,4-dicyanobenzene (DCB) (**12** to **21**). Aliphatic carboxylic acids, which generate highly energetic $C(sp^3)$ alkyl radicals without stabilization that often lead to catalyst decomposition in photochemical systems (*6*, *7*), were well tolerated under our μRN-eChem conditions (**12** to **14** and **17** to **19**, 49 to 66% yield). Benzylic carboxylic acids, including the anti-inflammatory drug

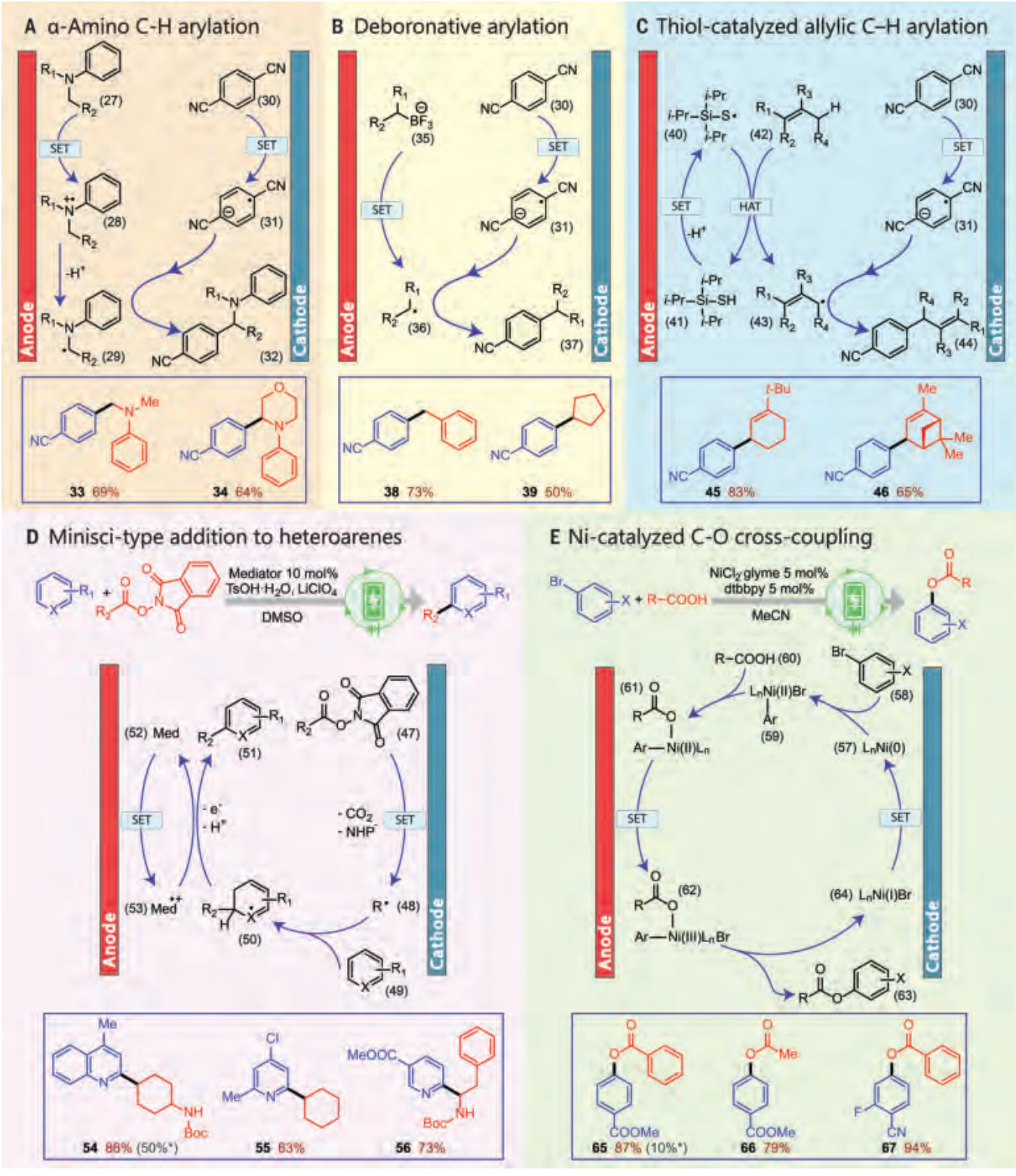

Fig. 3. General applicability of μRN-eChem platform for SET redox-neutral chemistry. (**A**) α-Amino C–H arylation. (**B**) Deboronative arylation. (**C**) Thiol-catalyzed allylic C–H arylation. HAT, hydrogen atom transfer; *i*-Pr, isopropyl. (**D**) Minisci-type radical addition to heteroarenes. Mediators (Med) used are ferrocene or 4-methoxytriphenylamine. Ts, tosyl; DMSO, dimethyl sulfoxide; NHP, phthalimide. (**E**) Ni-catalyzed C–O cross-coupling. dtbbpy, 4,4′-di-*tert*-butyl-2,2′-dipyridyl. Asterisks indicate high-performance liquid chromatography yield obtained from batch setup (fig. S5B) under identical electrochemistry conditions. See the supplementary materials for detailed reaction conditions.

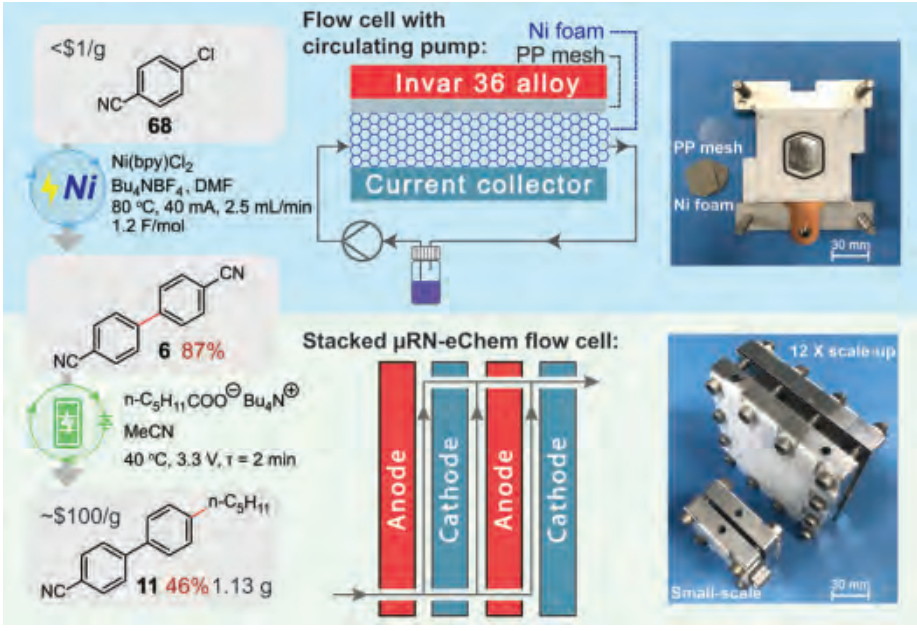

Fig. 4. A fully electrochemical two-step synthesis of liquid crystal material 5CB. See the supplementary materials for detailed reaction conditions. DMF, *N*,*N*′-dimethylformamide; PP, polypropylene.

naproxen (**16b**), could be smoothly transformed to 1,1-diarylalkanes (**15** and **16**, 65 and 73%, respectively). Additionally, 77% of unreacted **16b** (based on the quantity of starting material used) was separated, demonstrating the recovery of excess carboxylic acid. Benzoyl-protected α-amino acids, delivering α-amino radicals, can also serve as good coupling partners to produce benzylic amides (**20** and **21**, 70 and 71% yield, respectively). The scope of electron-deficient aryl nitriles has also been examined. An ester group was a suitable alternative to the nitrile electron-withdrawing group (**22**, 52% yield). The BPDN radical anion ($BPDN^{\bullet-}$) **7**, despite its relatively reduced stability compared with the DCB radical anion ($DCB^{\bullet-}$) (Fig. 1D and fig. S3), coupled with α-amino radical in high efficiency (**9**, 67%). Derivatives of DCB, including those bearing heterocycle substituents, were effective for radical-radical cross-coupling to give corresponding coupling products (**23** to **26**, 49 to 80% yield).

Given the successful implementation of μRN-eChem with decarboxylative arylation reactions, we posited that other transient radical precursors could react in a similar manner. We first tested an electrochemical α-amino C–H arylation reaction (Fig. 3A) (*26*). Anodic oxidation of amine **27** [$E_{p/2}$ = +0.50 V (versus Fc/Fc^+)] generates the radical cation **28**, and deprotonation of the methylene group α to the N gives α-aminoalkyl radical **29**, which undergoes cross-coupling with the cathodically generated persistent radical **31** to form arylation products (**33** and **34**, 69 and 64% yield, respectively). Electrochemical deboronation of trifluoroborate **35** [$E_{p/2}$ = +0.55 V (versus Fc/Fc^+)] is a similar process to anodic decarboxylation, yielding a $C(sp^3)$ alkyl radical **36** (*27*, *28*) (Fig. 3B). Radical-radical coupling of **31** and **36** with subsequent decyanation gave **38** and **39** with good efficiency (73 and 50%, respectively). This μRN-eChem radical-radical cross-coupling strategy can also function synergistically with other catalytic mechanisms. We demonstrated the thiol-catalyzed allylic arylation reaction as an example (Fig. 3C). Electrophilic thiyl radical **40**, generated from anodic oxidation of thiol **41** under basic conditions [$E_{p/2}$ = +0.41 V (versus Fc/Fc^+)], can regioselectively abstract an allylic hydrogen atom from alkene **42** to provide the allylic radical **43** (*29*). Rapid trapping of **43** using a persistent radical **31** forges a new $C(sp^2)$–$C(sp^3)$ bond (**45** and **46**, 83 and 65% yield, respectively).

In addition to the radical-radical cross-coupling, other types of SET redox-neutral transformations can also be implemented on our μRN-eChem platform. Recently, preactivated carboxylic acid *N*-hydroxyphthalimide esters have been used in photocatalyzed Minisci-type reactions to functionalize heterocycles, avoiding the high oxidation potential of directly activating carboxylic acids and thus improving functional group tolerance (*30*, *31*). In the presence of a redox mediator **52**, μRN-eChem is able to shuttle the electron between the anode and the transient radical **50** generated near the cathode, furnishing Minisci products in good yield (**54** to **56**, 63 to 86%) (Fig. 3D). Furthermore, μRN-eChem is compatible with SET redox-neutral transition-metal catalysis that has been extensively studied with photocatalysis (*32*). As an example, nickel-catalyzed $C(sp^2)$–O cross-coupling (*33*) was accomplished on the μRN-eChem platform to form O-aryl esters (**65** to **67**, 79 to 94% yield) (Fig. 3E). Mechanistically, the cathodic reduction of Ni(I) **64** to Ni(0) **57** facilitates the oxidative addition, and the anodic oxidation of Ni(II)(aryl) carboxylate **61** to Ni(III) **62** promotes the reductive elimination (*34*, *35*).

Compared with photochemistry, the catalyst-free, low-cost characteristics of μRN-eChem make it potentially more economical for synthesizing medium-value chemicals in addition to high-value fine chemicals (e.g., pharmaceuticals). We decided to demonstrate the practicality of μRN-eChem with the synthesis of 4-cyano-4′-pentylbiphenyl (5CB), a commonly used nematic liquid crystal material. Currently, 5CB is produced from biphenyl in a linear synthesis, which suffers from low yields and low efficiency (*36*). We proposed a fully electrochemical two-step synthesis to upgrade readily available 4-chlorobenzonitrile (4-ClBN) (<$1/g) to 5CB (~$100/g) with inexpensive reagents (Fig. 4). The first step, synthesis of BPDN, was a cathodic homocoupling of 4-ClBN catalyzed by $NiCl_2$ with 2,2′-bipyridine (bpy) as the ligand, and Invar 36 Fe/Ni alloy as the sacrificial anode. An electrochemical flow cell was engineered to handle gram-scale electrochemical synthesis (2.65 g synthesized in 24 hours) under air-free environment and elevated temperature, achieving an improved yield (87%) compared with a batch electrochemical setup (*37*). The second step used the μRN-eChem platform developed in this work to cross-couple BPDN and hexanoic acid under catalyst- and electrolyte-free conditions giving the targeted product (5CB). To demonstrate the scalability of μRN-eChem, we devised a three-layer stacked microfluidic flow cell (*38*, *39*), resulting in a 12-fold productivity increase relative to the small-scale flow cell. This scaled-up version continuously operated for 67 hours without intervention, yielding 1.13 g of 5CB.

The μRN-eChem strategy demonstrates that microfluidic technology and chemistry can work in tandem to controllably realize SET redox-neutral chemistry. Although the protocol uses an excess of one of the substrates, we anticipate that this conceptual approach will inspire the development of other redox-neutral electrosynthetic methods as a complement to existing redox-neutral photochemical synthesis technologies.

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SUPPLEMENTARY MATERIALS

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GEOPHYSICS

3D fault architecture controls the dynamism of earthquake swarms

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The vibrant evolutionary patterns made by earthquake swarms are incompatible with standard, effectively two-dimensional (2D) models for general fault architecture. We leverage advances in earthquake monitoring with a deep-learning algorithm to image a fault zone hosting a 4-year-long swarm in southern California. We infer that fluids are naturally injected into the fault zone from below and diffuse through strike-parallel channels while triggering earthquakes. A permeability barrier initially limits up-dip swarm migration but ultimately is circumvented. This enables fluid migration within a shallower section of the fault with fundamentally different mechanical properties. Our observations provide high-resolution constraints on the processes by which swarms initiate, grow, and arrest. These findings illustrate how swarm evolution is strongly controlled by 3D variations in fault architecture.

Earthquake swarms are enigmatic features of natural seismicity, with each sequence exhibiting a unique evolutionary pattern that is typically nonstationary in space and time. Swarms are frequently associated with regions of elevated heat flow (*1*, *2*) and are thought to be primarily driven by external processes such as fluids or aseismic slip (*3*–*5*) rather than earthquake triggering via stress transfer. The processes driving swarms are inferred from the observed diffusive or migratory evolutionary patterns and the tendency for the largest events to occur substantially after the onset of the sequence (*6*). However, much of the dynamism of swarms remains unexplained and likely reflects our limited knowledge of the structural and permeability architecture of the fault zones that produce them.

Fault zones are multiscale systems consisting of a localized fault core that accommodates the primary slip (*7*) and surrounding damage zones that contain highly fractured material. Damage zones commonly exhibit a fracture density that decays exponentially with distance from the fault core (*8*) and may contain additional smaller embedded structures (*9*). Fault zones have an appreciable influence on fluid flow in the crust, with higher-permeability damage zones acting as channels for fluids, whereas the fault core can act as either a conduit or a barrier to fluid flow (*10*, *11*). The permeability architecture of fault zones may be anisotropic (*12*) and transient (*13*, *14*), with geometric complexities sometimes creating secondary permeability structures in fault zones that further control fluid behavior (*15*).

Models for general fault architecture are predominantly compiled from geological and hydrological measurements obtained near the surface. Exhumed faults have advanced our understanding of the internal structure of fault zones at seismogenic depths (*7*, *16*) but provide limited constraints on their full three-dimensional (3D) variations. Occasionally, samples of fault material are obtained via drilling (*17*), which provide valuable but sparse constraints on deeper fault properties. Additionally, studies in mines provide glimpses of the complex faults and fracture surfaces that can occur at depth (*18*). These laterally heterogeneous structures are often generalized to three dimensions by assuming they vary smoothly with depth. However, recent seismological studies have increasingly observed that fault zones are highly 3D systems with complex depth-dependent variations (*19*, *20*), numerous strands over many scales (*21*, *22*), and intricate spatially variable damage structures (*23*). These observations provide insights into the connections between fault structure and earthquake physics, yet gaps remain in understanding deep fault structure and permeability architecture, which is essential for understanding the complex evolution of earthquake swarms.

From early 2016 to late 2019, a long-term, vigorous swarm occurred near Cahuilla, California, roughly halfway between the San Jacinto and Elsinore fault zones (*24*) (Fig. 1). The swarm likely occurred in the Cahuilla Valley pluton, a hornblende-biotite tonalite that is the predominant intrusive rock in the area (*25*). To better understand the sequence and properties of the fault zone, we used earthquake detection algorithms with deep neural networks to produce a highly detailed seismicity catalog of more than 22,000 events with moment magnitudes ranging from 0.7 to 4.4 (*26*). We discovered a fault zone with rich 3D structure that controls the dynamic evolution of the swarm.

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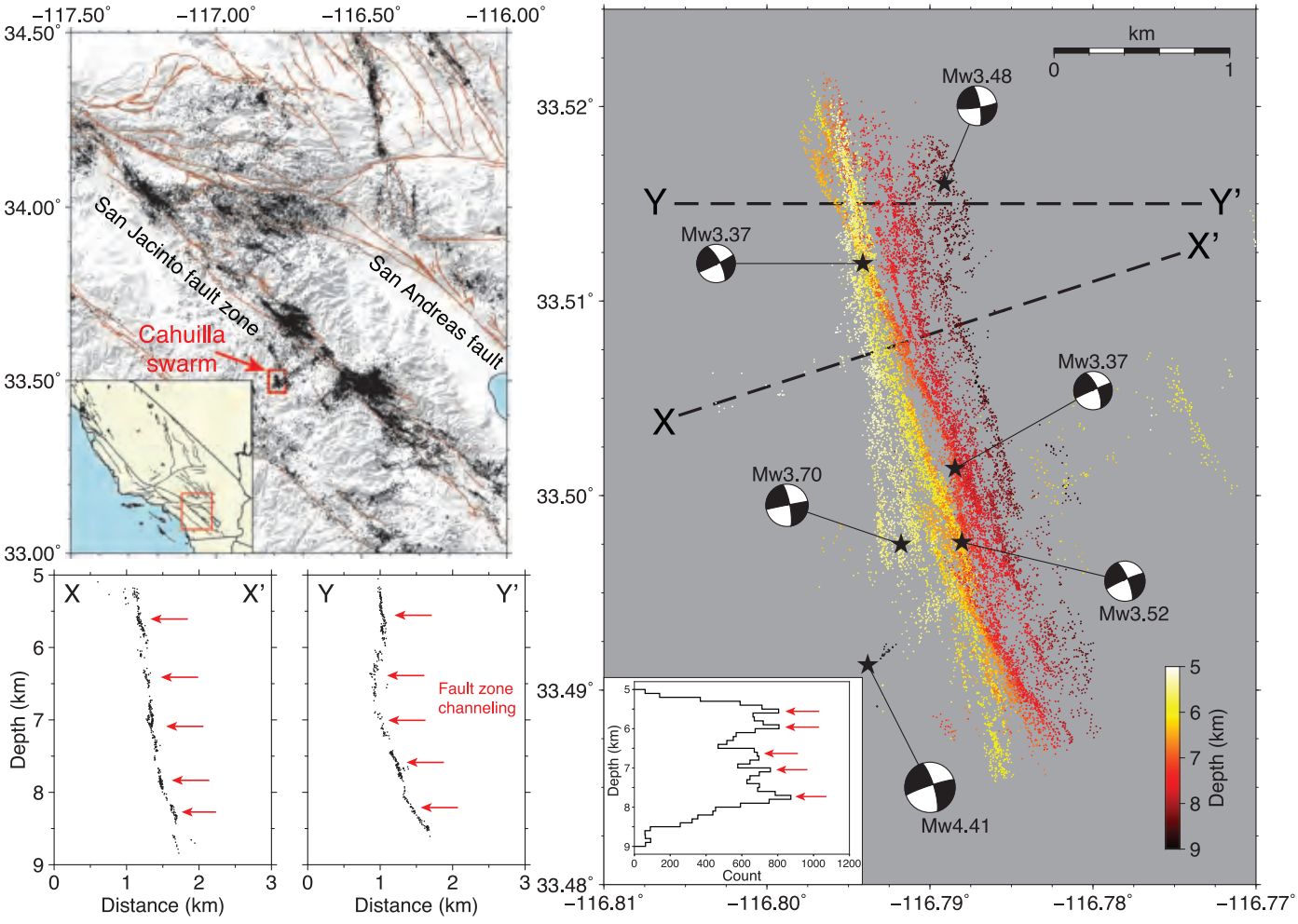

Fig. 1. Relocated seismicity during the Cahuilla swarm. Fault zone is less than 50 m wide and exhibits prominent channeling along strike. Cross sections X-X' and Y-Y' show that there is considerable curvature to the fault geometry over short distances.

A high-resolution perspective of the 3D geometry of the fault zone is observed in the relocated seismicity. The swarm occurred in a single nonplanar right-lateral strike-slip fault surface that dips 70° to 80° toward the northeast (Fig. 1). In the fault-normal direction, the seismicity is just tens of meters thick, which is comparable to the relative horizontal location errors (38 m); the seismogenic portion of the fault zone may, therefore, be even narrower. The fault geometry is nonplanar both along strike and along dip, most notably at the northwestern edge, where the fault surface undergoes multiple reversals in the dip direction as a function of depth.

Within the fault zone, there is a stack of elongated strike-parallel channels of relatively high seismicity density with vertical separation of ~200 to 400 m. This distance is larger than the relative vertical error (87 m). These channels have a weakly undulating geometry and persist over most of the fault zone. They are also prominently observed in the aggregate distribution of focal depths (Fig. 1), where they are represented by five peaks.

The 3D fault geometry is critical for understanding the spatiotemporal evolution of the swarm (Fig. 2). We can trace the origin of the sequence to a 100-m–wide region near the base of the fault zone (8-km depth). The swarm expanded slowly in the fault zone over several years, ultimately extending across an area that measured 4 km by 4 km. We observed a prominent front to the migration, with sustained earthquake activity behind it (Fig. 2). However, virtually no earthquake triggering occurs ahead of the front, which is consistent with expectations for a sustained point injection source (*27*). By interpreting the migration of seismicity as due to fluid flow, the slow diffusion front suggests that the swarm is driven predominantly by fluid migration (*28*) rather than static or dynamic triggering, as is more typical of earthquake sequences. The migration speeds are extremely slow at 1 to 5 m/day, which is much slower than the propagation speed of slow slip events (tens of kilometers per day) (*3*) and also slower than many natural injection-driven swarms (*29*). We estimate the permeability of this fault zone to be ~10^{-17} to 10^{-18} m^2 (*26*). The observations bear similarities to earthquake sequences induced by deep fluid disposal (*27*), although here fluid injection is occurring directly into the fault zone from a deep, natural source.

The spatial expansion of the swarm is not uniform, indicating that the permeability architecture of the fault zone is variable (Fig. 2, A and B). There may be a permeability barrier 0.5 km below the injection source at around 8-km depth, as only a minimal amount of deeper seismicity occurred during the nearly 4 years of activity. This may alternatively indicate that the fault zone itself does not extend to greater depths or that there was a change in rheology at this point. Laterally, the swarm was constrained on both sides after expanding to an along-strike length of 4 km, which was reached ~3 years into the sequence. This lateral expansion was asymmetric, migrating ~1.8 times faster to the northwest than to the southeast. Further expansion may be limited by permeability barriers, or the fault zone itself may not extend beyond the final spatial extent of the sequence.

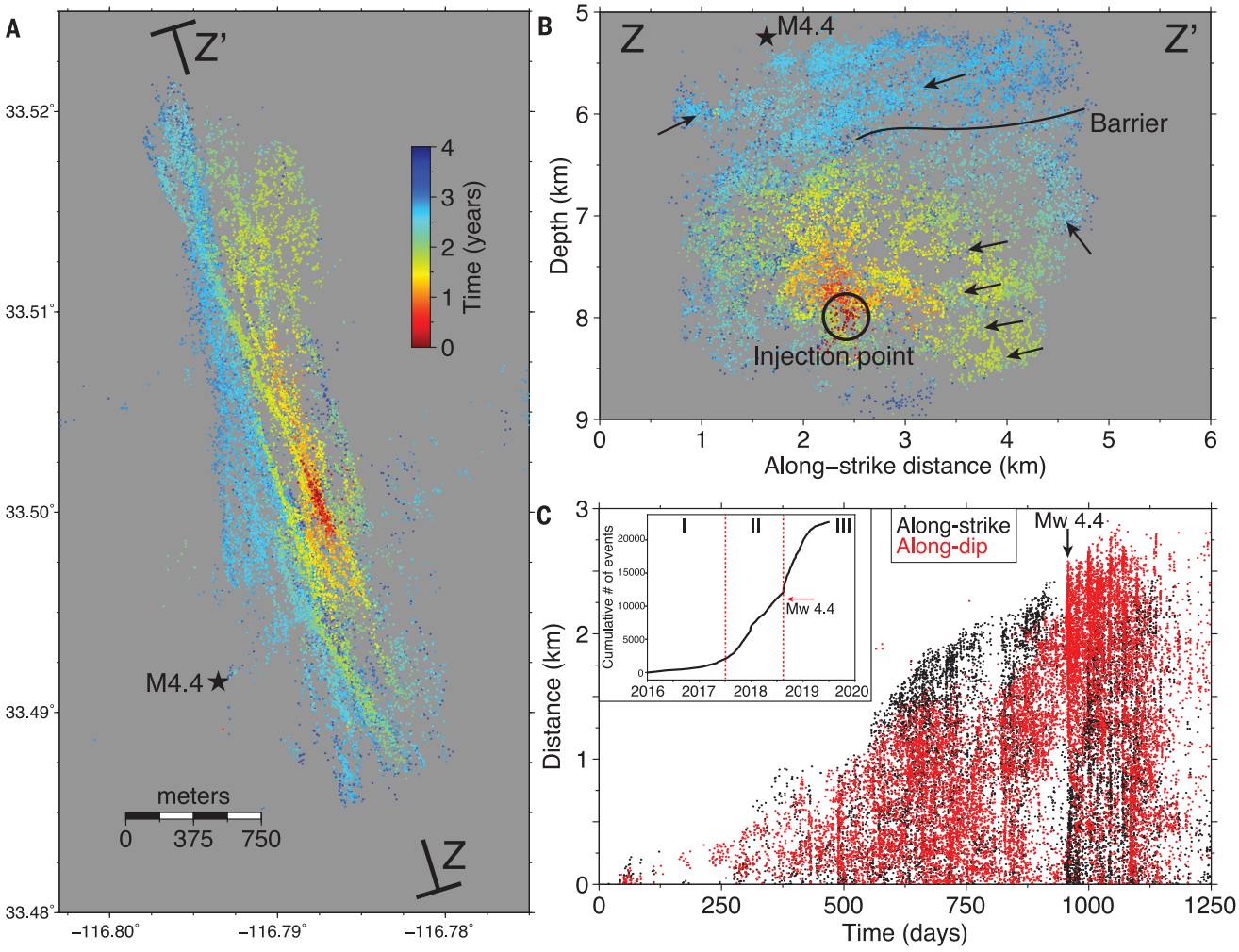

Fig. 2. Spatiotemporal evolution of the sequence. Fluids are naturally injected into the base of the fault zone and diffuse within it through channels (black arrows). (**A**) Map view of seismicity evolution, with events color-coded by occurrence time. (**B**) Fault-parallel cross section along Z-Z', including the inferred permeability barrier and injection point. (**C**) Absolute distance of seismicity relative to injection point in (B) projected into along-strike and along-dip components.

Fluid pressures may also no longer be high enough to produce additional events. In the fault-normal direction, the swarm is limited to a few tens of meters in width, with no evidence of expansion (Fig. 1). Our observations are consistent with models of fault architecture that have a damage zone embedded in relatively impermeable host rock (*11*).

From 2016 to mid-2017, the migration velocity of the swarm was roughly isotropic (Fig. 2C). Then, the along-strike component of the migration accelerated relative to the along-dip component, which coincided with the fluids entering the fault channels.

In August 2018, nearly 3 years after the swarm's onset, the up-dip progression stalled at a depth of 6 km across most of the fault zone (Fig. 2B). Careful examination of the seismicity indicates that up-dip migration still continues, but only through a 1-km-long strike-parallel aperture near the southeast end of the fault zone (Fig. 2B). After the localized events migrated upwards for 500 m, seismic activity escalated rapidly and eventually culminated in the largest earthquake of the sequence, a moment magnitude (M_w) 4.4 event. The second-largest event to precede the M_w 4.4 mainshock was a M_w 2.8 earthquake that occurred 4 days earlier. After the mainshock, the swarm migrated almost unilaterally to the northwest, filling a gap in seismicity at depths less than 6 km. From these observations, we infer a 2-km-long subhorizontal permeability barrier at 6-km depth, with limited to no fluid flow across it. This barrier correlates with a dip reversal of the fault zone (Fig. 1, Y-Y′). Thus, permeability structures in the fault zone exert a major control on the local diffusion properties.

Approximately 850 days into the sequence, the fluid injection may stop, as evidenced by the lack of seismicity within 1 km of the injection source, and swarm migration continues at further distances (Fig. 2C).

The M_w 4.4 earthquake denotes a key transition point in the swarm. The sequence initiated with steady but low seismicity rates from 2016 to 2017, followed by somewhat higher rates until the occurrence of the M_w 4.4 earthquake (Fig. 2C, periods I and II). Immediately after the M_w 4.4 earthquake, the seismicity rate increased substantially (Fig. 2C, period III) but then decayed, with the swarm eventually dying out about a year later. The seismicity rate throughout period III was much more typical for earthquake swarms than the steady rate observed in periods I and II, as there is stronger time dependence and temporal clustering (*6*).

The distinctly different character of periods I and II compared with period III leads to the question of what physical processes control the event rate behavior. The simplest explanation is that during periods I and II, the swarm was driven predominantly by fluid pressure diffusion through the fault zone, whereas during period III, the swarm was driven by a mixture

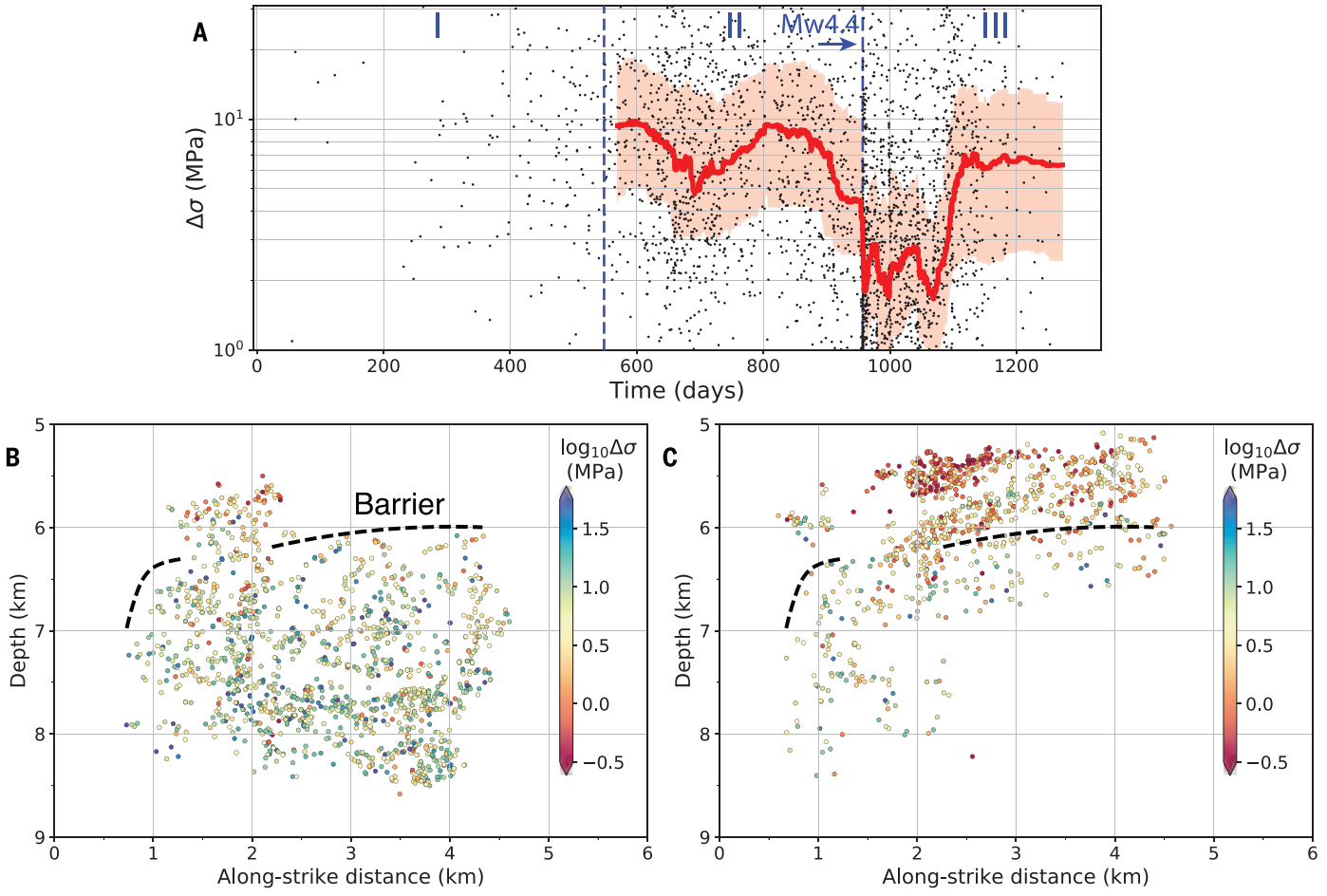

Fig. 3. Evolution stress drop (Δσ). (**A**) Time evolution. The solid red line marks the median Δσ in a causal, 200-event moving window. Shading denotes the interquartile range. (**B**) Along-strike cross-sectional view of events occurring before the M_w 4.4 earthquake. Bluer colors indicate events that are enriched in high-frequency energy. Δσ decreases as the swarm circumvents the barrier at 6-km depth. (**C**) Along-strike cross-sectional view of events occurring in the first 50 days after the M_w 4.4 earthquake. For events shallower than 6 km, Δσ is an order of magnitude lower.

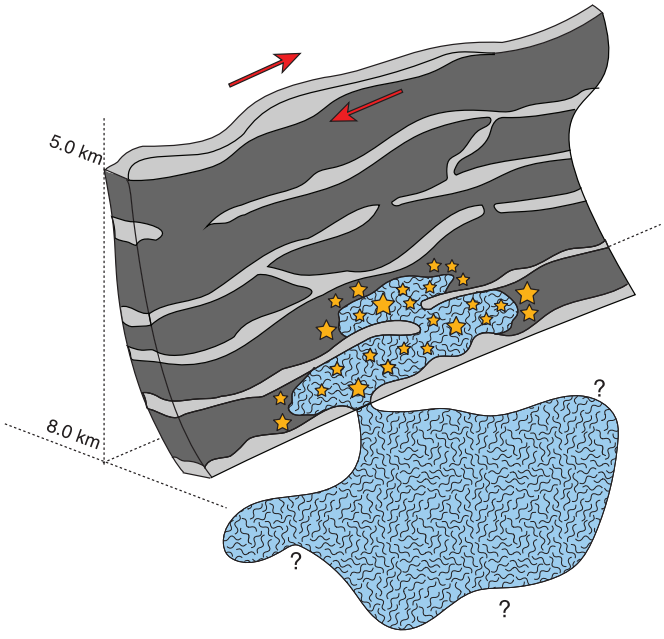

Fig. 4. Cartoon summarizing observations. A fault zone is embedded in host rock and exhibits geometric warping along strike and along dip. Within the fault zone, there are undulating subhorizontal channels. A small portion of the base of the fault zone connects to a deeper reservoir that is initially sealed. Once the seal breaks, fluid is injected into the fault zone, which diffuses primarily through channels, driving the seismicity. During the nearly 4 years of activity, fluids interact with numerous permeability barriers.

of static stress changes and fluid pressure changes. From Fig. 2C, periods I and II exhibit no triggering ahead of the front and little clustering, whereas period III has pervasive clustering. The M_w 4.4 earthquake produced appreciable stress changes on the surrounding fault zone that may have altered the permeability structure of the damage zone, possibly enhancing fluid flow through the system.

We examined earthquake stress drops (Δσ) in the context of the structural architecture of the fault zone and sequence evolution. Stress drop is a fundamental earthquake source parameter and a key component of the seismic energy budget (*30*). We derived Δσ for earthquakes with local magnitude (M_L) > 0.5 from a spectral decomposition approach (*26*). Figure 3A shows the median Δσ in a sliding window over the duration of the sequence. Throughout most of period II, the median stress drop fluctuates around 8 MPa (Fig. 3A) before dropping markedly to a range of 2 to 3 MPa. The

median $\Delta\sigma$ remains low for ~5 months before a stepwise recovery to a value that approaches the pre-mainshock level.

Before the occurrence of the M_w 4.4 earthquake (period II), the swarm located almost entirely below 6-km depth, and the $\Delta\sigma$ values were generally in the range 4 to 15 MPa (median ~8 MPa) (Fig. 3B). As the fluids circumvented the barrier at 6-km depth, coincident with the M_w 4.4 earthquake, the median values of $\Delta\sigma$ showed a prominent step decrease to around 2 MPa. Fluids then moved horizontally through a channel toward the northwest (up-dip of the 6-km barrier), and in this channel, $\Delta\sigma$ values were persistently lower (1 to 5 MPa). The $\Delta\sigma$ of earthquakes down-dip of the 6-km depth barrier during this time were in the same range as they were before the M_w 4.4 earthquake. The pronounced change in median $\Delta\sigma$ at 6-km depth, along with the inferred permeability barrier at the same place, suggests a sharp interface between materials with fundamentally different structural properties.

We used deep-learning algorithms to produce detailed images of fault zone structure at seismogenic depths, which reveal a system with permeability that is highly variable in three dimensions. In particular, we resolve strike-parallel channeling in the fault zone that controls the migration of events. These observations may relate to channeling seen in numerical and laboratory experiments of flow in fault zones and fractures (*31*, *32*), or to anisotropic fault roughness (*33*). We also resolve a subhorizontal permeability barrier that, when circumvented, allows for the largest earthquake in the sequence. Together, these factors illustrate how the 3D permeability structure of the fault zone controlled the evolution of the Cahuilla swarm (Fig. 4).

Standard models for fault zone hydrogeology mainly distinguish between fault-parallel and fault-normal flow (*11*) and how these systems act as conduits and/or barriers to flow depending on the geometrical aspects of the damage zone. Although our results provide evidence in support of these models, particularly at seismogenic depths, for which previous observations are lacking, they also highlight limitations in generalizing fault architecture to three dimensions from near-surface observations alone.

The prominent diffusive character of the swarm relative to a highly localized injection point suggests that the fault zone connects to a reservoir at depth but was sealed initially. This scenario is described by the fault valve model (*34*), in which a permeability seal at the roots of a fault zone is suddenly broken, allowing fluid to be transported up-dip through the fault zone via an overpressured hydraulic gradient. Fault valving has been invoked to explain the activation of faults under various circumstances (*19*, *29*, *35*), but most of the evidence for this phenomenon has been circumstantial. Our observations provide clear evidence of fault valving as a mechanism for seismogenesis in swarms. This is noteworthy because understanding how and why earthquake sequences initiate is among the greatest outstanding challenges in earthquake science. Because two nearby swarms in the 1980s also exhibited migration patterns over several years (*24*), it is likely that this reservoir has a large spatial extent and connects to many faults in the area, making this a key mechanism for transporting fluids through the crust.

Permeability is not a static, scalar property but instead a tensor that may vary in space and time. The Cahuilla swarm neatly illustrates this point, as the spatiotemporal evolution of seismicity is quite different from what would be observed if permeability were constant and isotropic. Permeability is expected to evolve with time in response to slip and fault healing processes, a fact that has important implications for earthquake cycle dynamics (*29*, *36*). Improved observational constraints of these processes as they occur in real fault systems will, therefore, help inform our understanding of earthquake physics. Measurements of earthquake stress drop commonly exhibit variability of 3 to 4 orders of magnitude (*37*), and understanding how much of this is natural (rather than due to error) is important because the variability can be related back to the intrinsic structural properties of fault zones or extrinsic factors such as stress. We observe a sudden change of nearly an order of magnitude in the median stress drops of events in the upper 1 km of the sequence. By having detailed knowledge of the internal structure of the fault zone that produced the Cahuilla swarm, we conclude that the fault is divided vertically into two sections with different mechanical properties that are separated by a permeability barrier.

Earthquake science has always been challenged by the staggering evolutionary complexity observed within and between different sequences. This complexity has made it difficult to distill common characteristics and formulate generalized conclusions that can provide constraints on the physics of earthquakes. With improving resolution, however, we are able to observe that sequence complexity often arises from the 3D characteristics of fault zones, which are themselves dynamic.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6497/1357/suppl/DC1
Materials and Methods
Figs. S1 to S6
References (*39*–*54*)

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CORONAVIRUS

Rapid implementation of mobile technology for real-time epidemiology of COVID-19

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The rapid pace of the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents challenges to the robust collection of population-scale data to address this global health crisis. We established the COronavirus Pandemic Epidemiology (COPE) Consortium to unite scientists with expertise in big data research and epidemiology to develop the COVID Symptom Study, previously known as the COVID Symptom Tracker, mobile application. This application—which offers data on risk factors, predictive symptoms, clinical outcomes, and geographical hotspots—was launched in the United Kingdom on 24 March 2020 and the United States on 29 March 2020 and has garnered more than 2.8 million users as of 2 May 2020. Our initiative offers a proof of concept for the repurposing of existing approaches to enable rapidly scalable epidemiologic data collection and analysis, which is critical for a data-driven response to this public health challenge.

The exponentially increasing number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections has led to "an urgent need to expand public health activities in order to elucidate the epidemiology of the novel virus and characterize its potential impact" (*1*). Understanding risk factors for infection and predictors of subsequent outcomes is crucial to gain control of the coronavirus disease 2019 (COVID-19) pandemic (*2*). However, the speed at which the pandemic is unfolding poses an unprecedented challenge for the collection of exposure data to characterize the full breadth of disease severity, hampering efforts for timely dissemination of accurate information to affect public health planning and clinical management. Thus, there is an urgent need for an adaptable real-time data-capture platform to rapidly and prospectively collect actionable high-quality data that encompass the spectrum of subclinical and acute presentations and identify disparities in diagnosis, treatment, and clinical outcomes. Addressing this priority will allow for more accurate estimates of disease incidence, inform risk mitigation strategies, facilitate allocation of scarce testing resources, and encourage appropriate quarantine and treatment of those afflicted.

An evolving body of literature suggests that COVID-19 incidence and outcomes vary according to age, sex, race, ethnicity, and underlying health status, with inconsistent evidence suggesting that commonly used medications—such as angiotensin-converting enzyme inhibitors, thiazolidinediones, and ibuprofen—may alter the natural disease course (*3*–*9*). Further, symptoms of COVID-19 vary widely, with fever and dry cough reportedly the most prevalent, though numerous investigations have demonstrated that asymptomatic carriage is a significant determinant of community spread (*5*–*7*, *10*–*13*). In addition, the full spectrum of clinical presentation, which is still being characterized, may differ markedly across patient subgroups. Recent advisories from the American Gastroenterological Association, the American Academy of Otolaryngology–Head and Neck Surgery, and the British Geriatrics Society have emphasized the potential association between COVID-19 infection and previously underappreciated gastrointestinal symptoms (e.g., nausea, anorexia, and diarrhea), loss of taste and/or smell, and common geriatric syndromes (e.g., falls and delirium). The pandemic has considerably outpaced our collective efforts to fully characterize who is most at risk or may suffer the most serious sequelae of infection.

Mobile phone applications and web-based tools facilitate self-guided collection of population-level data at scale (*14*), the results of which can be rapidly redeployed to inform participants of urgent health information (*14*, *15*). Both technologies are particularly advantageous when many individuals are advised to maintain physical distance from others (*16*). Such digital tools have already been applied in more controlled research settings, and these studies benefit from greater lead time for field testing, question curation, and recruitment. Although many digital collection tools for COVID-19 are being developed and launched in the Unites States and abroad (see http://mhealth-hub.org/mhealth-solutions-against-covid-19 for a continuously updated resource list from the European Union and the World Health Organization), including some in partnership with government health agencies such as the Centers for Disease Control and Prevention, most applications have largely been configured to offer a single assessment of symptoms to tailor semipersonalized recommendations for further evaluation. Infectious disease surveillance web-based tools (e.g., http://flunearyou.org) have been rapidly adapted for COVID-19–specific collection (e.g., http://covidnearyou.org). Alternatively, web portals have been developed for researchers to report patient-level information on behalf of participants already enrolled in clinical registries (e.g., ccc19.org). Integration with approaches that use remote data capture (e.g., wearable technology or symptom checkers such as real-time reporting thermometers) is also being considered. Although these approaches offer critical public health insights, they are often not tailored for the type of scalable longitudinal data capture that epidemiologists need to perform comprehensive, well-powered investigations.

To meet this challenge, we established a multinational collaboration, the COronavirus Pandemic Epidemiology (COPE) Consortium, composed of leading investigators from several large clinical and epidemiological cohort studies. COPE brings together a multidisciplinary team of scientists with expertise in big data research and translational epidemiology to investigate the COVID-19 pandemic in a large and diverse patient population. Several large cohorts have already agreed to join these efforts, including the Nurses' Health Study series, the Growing Up Today Study (GUTS), the Health Professionals Follow-Up Study (HPFS), TwinsUK, American Cancer Society Cancer Prevention Study 3 (CPS-3), the Multiethnic Cohort Study, the California Teachers Study (CTS), the Black Women's Health Study (BWHS), the Sister Study, Aspirin in Reducing Events in the Elderly (ASPREE), the Stanford Nutrition Studies, the Gulf Long-term Follow-up (GuLF) Study, the Agricultural Health Study, the National Institute of Environmental Health Sciences Environmental Polymorphisms Registry, the Predicting Progression of Developing Myeloma in a High-Risk Screened Population (PROMISE) study, and the Precursor Crowdsourcing (PCROWD) study. To aid in our data harmonization efforts in the United States, we developed the COVID Symptom Study (previously known as the COVID

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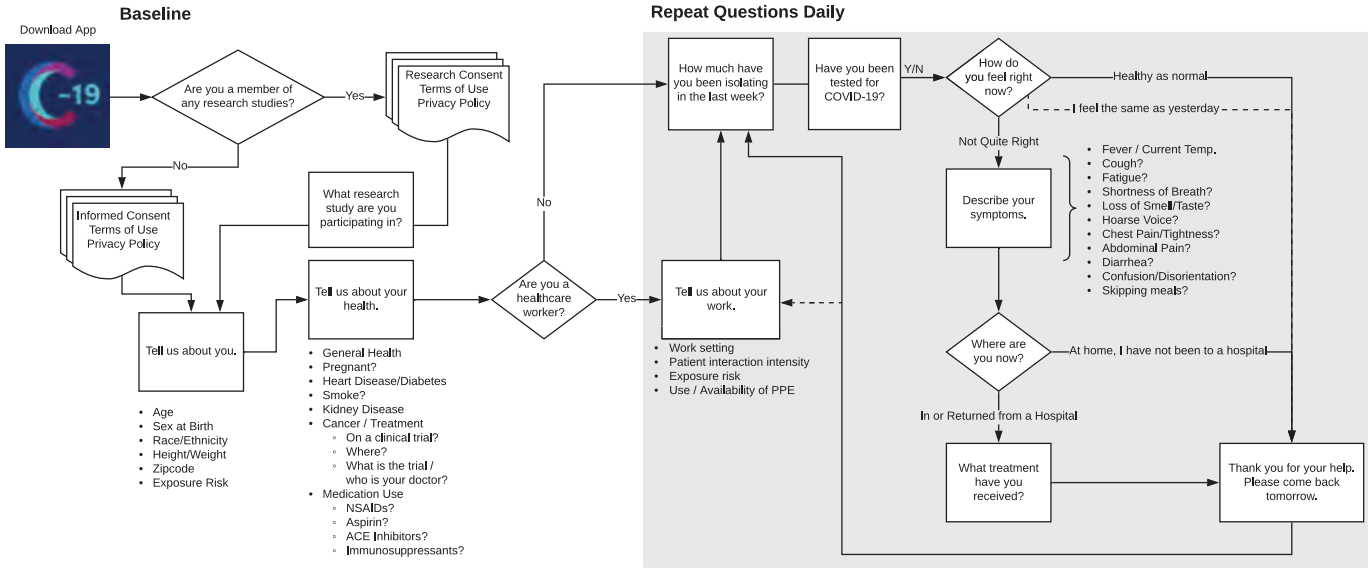

Fig. 1. Schematic of the participant workflow. After downloading the COVID Symptom Study app and providing consent, users are prompted to enter baseline demographic and clinical information and are serially queried about new or ongoing symptoms, testing results, and extent of isolation. Health care workers offer additional information about the intensity of their patient interactions, potential exposure to infected patients, and use of PPE. With informed consent, users also participating in a variety of ongoing cohorts or clinical trials (Nurses' Health Study, TwinsUK, and others) have the option of linking COVID Symptom Study information to their extant research data.

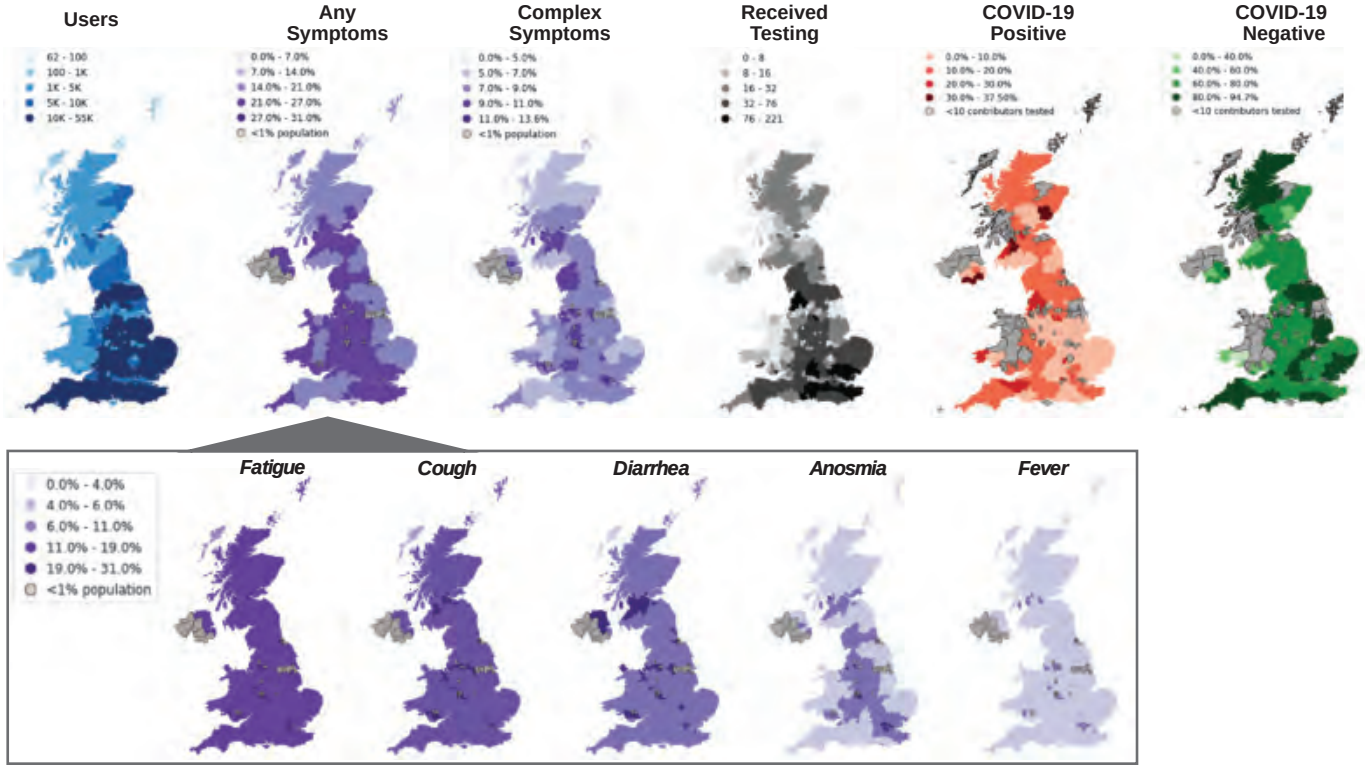

Fig. 2. COVID Symptom Study use, reported symptoms, and testing results according to geographic location in the United Kingdom. Between 24 and 29 March 2020, more than 1.6 million unique individuals downloaded the application and shared clinical and demographic information, as well as daily symptoms and high-intensity occupational exposures (blue map). Population density of those presenting with any symptoms (left purple map) varied according to region, with widespread reports of fatigue, cough, and diarrhea, followed by anosmia and, relatively infrequently, fever (inset). Examination of individuals who reported complex symptoms (right purple map), defined as having cough or fever and at least one other symptom (diarrhea, anosmia, or fever), reveals areas that potentially need more testing. For the subset of the population that received a COVID-19 test (black map), areas with larger proportions of positive tests (orange map) appear to coincide with areas in which high proportions of the population reported complex symptoms. By contrast, some areas with low prevalence of complex symptoms have received higher rates of testing and, consequently, more negative tests (green map). This example of real-time visualization of data captured by the COVID Symptom Study may help public health and government officials reallocate resources, identify areas with unmet testing needs, and detect emerging hotspots.

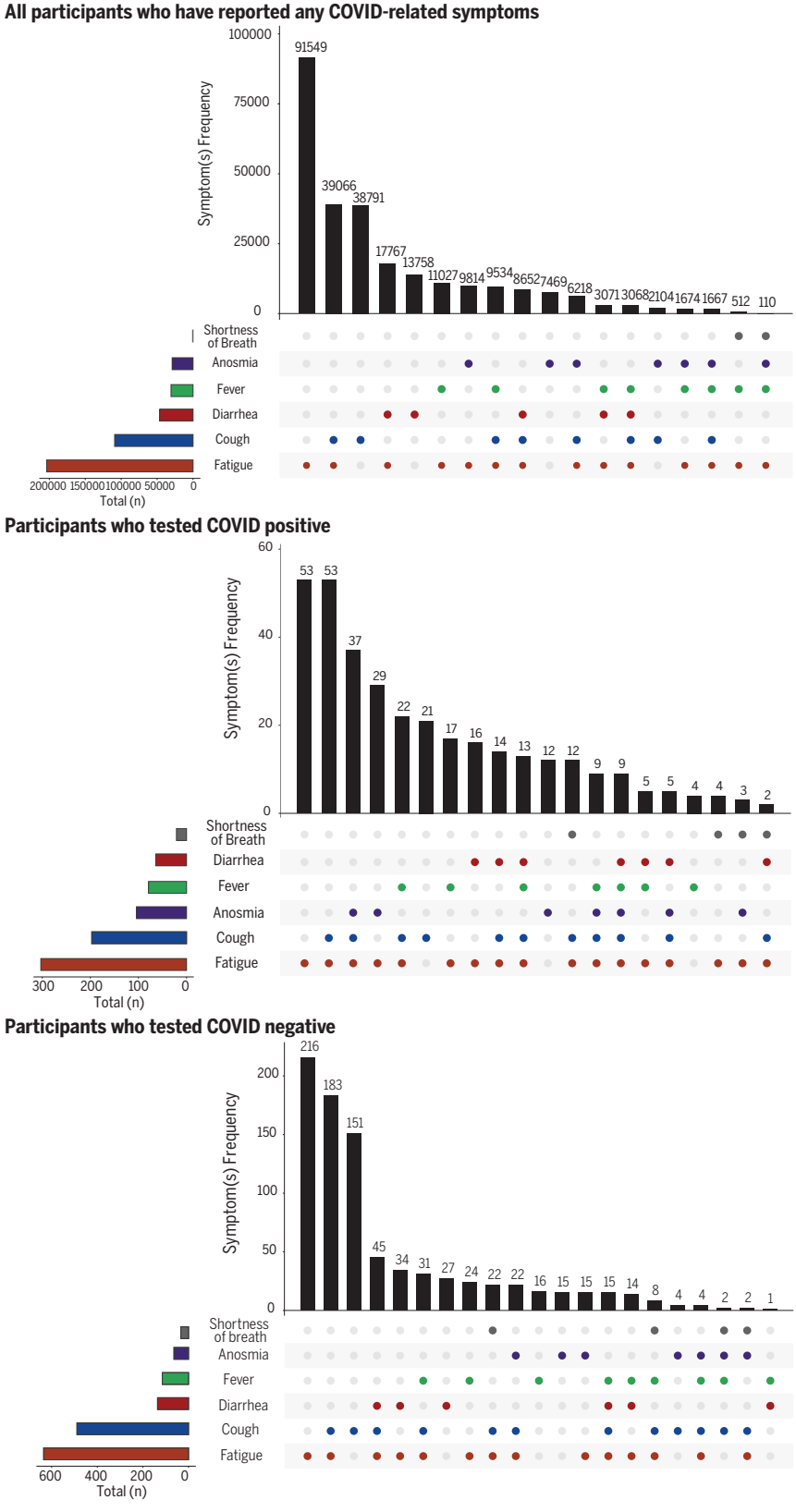

Fig. 3. Symptoms reported through the COVID Symptom Study app. By 27 March 2020, 265,851 individuals in the United Kingdom reported any symptom potentially associated with COVID-19 (**top**). Participants provided data on whether they were tested for COVID-19, as well as the test result. 1176 individuals reported having received a COVID-19 test (0.4% of those with symptoms). Symptom frequencies among those who tested positive (**middle**; n = 340) versus negative (**bottom**; n = 836) are shown.

Symptom Tracker) mobile app with in-kind contributions from Zoe Global Ltd., a digital health care company, and academic scientists from Massachusetts General Hospital and King's College London. By leveraging the established digital backbone of an application used for personal nutrition studies, the COVID Symptom Study app was launched in the United Kingdom on 24 March 2020 and became available in the United States on 29 March 2020 (https://covid.joinzoe.com/us). The COPE Consortium is committed to the shared international pursuit of combating COVID-19 and has worked with scientific collaborators and thought leaders in real-time epidemiology to prioritize data harmonization and sharing as part of the Coronavirus Census Collective (*17*).

The COVID Symptom Study enables self-reporting of data related to COVID-19 exposure and infections (Fig. 1). On first use, the app queries location, age, and core health risk factors. Daily prompts query for updates on interim symptoms, health care visits, and COVID-19 testing results. For those self-quarantining or seeking health care, the level of intervention and related outcomes are collected. Individuals without obvious symptoms are also encouraged to use the app. Through pushed software updates, we can add or modify questions in real time to test emerging hypotheses about COVID-19 symptoms and treatments. Notably, participants enrolled in ongoing epidemiologic studies, clinical cohorts, or clinical trials can provide informed consent to link survey data collected through the app to their preexisting study cohort data and any relevant biospecimens in a Health Insurance Portability and Accountability Act (HIPAA)–compliant and General Data Protection Regulation (GDPR)–compliant manner. A specific module is also provided for health care workers to determine the intensity and type of their direct patient care experiences, the availability and use of personal protective equipment (PPE), and work-related stress and anxiety.

Through rapid deployment of this tool, we can gain key insights into population dynamics of the disease (Fig. 2). By collecting participant-reported geospatial data, highlighted as a critical need for pandemic epidemiologic research (*15*), we can rapidly identify populations with highly prevalent symptoms in regions that may emerge as outbreak hotspots. An early snapshot of the first 1.6 million users in the United Kingdom over the first 5 days of use confirms the variability in symptoms reported across suspected COVID-19 cases and is useful for generating and testing broader hypotheses. At the time, users had a mean age of 41, ranged from 18 to 90 years old, and were 75% female. Graphic visualization of our initial results (Fig. 3) demonstrates that among those reporting symptoms by 27 March 2020 (n =

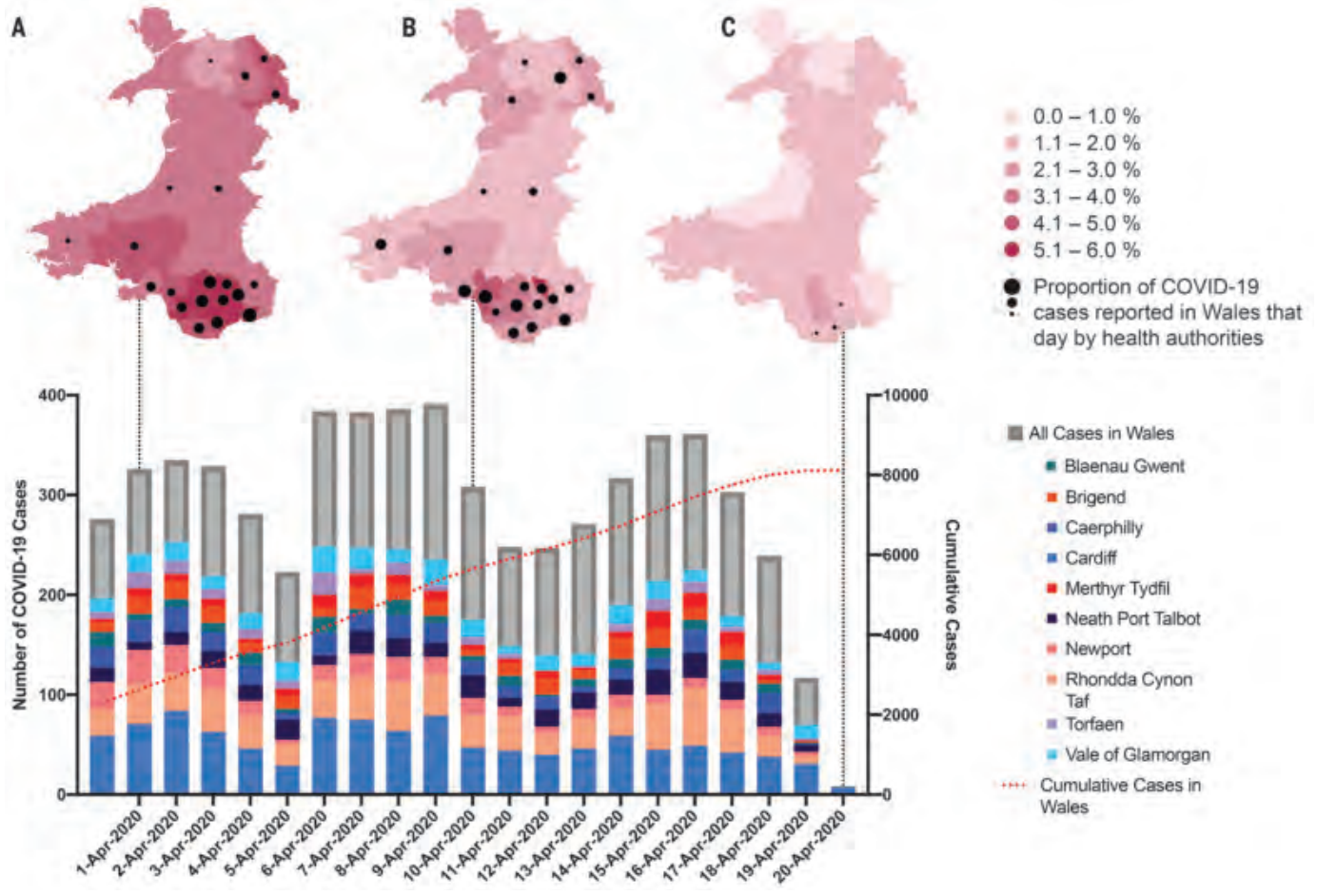

Fig. 4. Predicting COVID-19 cases on the basis of real-time symptom reporting in Wales, United Kingdom. This time series (bar graph) displays the number of new confirmed cases (gray bars) reported by the Public Health Wales NHS Trust between 31 March 2020 and 20 April 2020. After 2 April, case numbers appear to have declined through 5 April. However, our symptom-based prediction model (*18*), developed from symptom reports from untested users of the COVID Symptom Study app, showed a high proportion of predicted COVID-19 cases in southern Wales on 1 April [dark red areas in (**A**)]. Six days later, Welsh health authorities reported a subsequent peak in cases over a 4-day period (6 to 9 April), driven primarily by these southern regions (colored bars). By 10 April, new confirmed cases across Wales declined. However, on the basis of reported symptoms (**B**), regions in South Wales still had high predicted levels of COVID-19, which became apparent as a second spike in confirmed cases between 15 and 16 April. As of 20 April (**C**), predicted COVID-19 prevalence across Wales according to symptom reporting appears to be low, which corresponds to a flattening of the cumulative incidence curve. However, several regions in southern Wales still have relatively high reports of symptoms and appear at risk for subsequent cases of COVID-19. Black dots on the maps represent the relative proportion of positive tests reported by health authorities across Wales that day by region. The prediction mapping included data from 1,339,670 users of the COVID Symptom Study on 1 April; 998,244 users on 10 April; and 1,234,918 users on 20 April. Public Health Wales NHS Trust data were current as of 21 April 2020 at 13:00 local time, taken from the "Rapid COVID-19 Virology - Public" dashboard (accessed via https://phw.nhs.wales/), and downloaded on 22 April 2020 at 12:30 p.m. Eastern Standard Time.

265,851 individuals), the most common symptoms were fatigue and cough, followed by diarrhea, fever, and anosmia. Shortness of breath was reported relatively rarely. Only 0.4% (n = 1176) of individuals reporting possible COVID-19 symptoms reported receiving a quantitative polymerase chain reaction test for COVID-19.

A comparison of symptomatic users who reported receiving a test within the initial launch period generated several hypotheses for future study with the growing dataset. The frequency of cough or fatigue alone or in combination commonly led to testing but was not a particularly accurate predictor of a positive test. Similarly, no individuals who reported diarrhea in the absence of other symptoms tested positive. Notably, more complex presentations with cough and/or fatigue and at least one additional symptom, including less commonly appreciated complaints such as diarrhea and anosmia, appeared to be enriched among those with positive test results relative to those with negative results. In particular, anosmia may be a more predictive symptom, as it was more common than fever in individuals who tested positive. Indeed, in subsequent analyses with a larger sample set, we have shown that anosmia appears to be a strong predictor for COVID-19 (*18*). By contrast, fever alone was not particularly discriminatory. However, when fever was present in combination with less appreciated symptoms, a greater frequency of positive tests was observed. These findings suggest that perhaps individuals with complex or multiple-symptom (three or more) presentations should be prioritized for testing. Concerningly, 20% of individuals reported complex symptoms

(cough and/or fatigue plus at least one of anosmia, diarrhea, or fever) but had not yet been tested, representing a substantial population that appears to be at elevated risk for the disease. Additional work is warranted to confirm whether complex or multiple-symptom cases can accurately predict COVID-19 incidence.

Building on these initial findings, our team subsequently developed a weighted prediction model based on the symptoms of more than 2 million individual app users (*18*). By using this prediction model, we demonstrate the potential utility of the COVID Symptom Study app to collect data for long-term studies as well as for immediate public health planning. In southern Wales in the United Kingdom, users reported symptoms that predicted, 5 to 7 days in advance, two spikes in the number of confirmed positive COVID-19 cases reported by public health authorities (Fig. 4). Conversely, a decline in reports of symptoms preceded a drop in confirmed cases by several days. These results demonstrate that this app prospectively captures the dynamics of COVID-19 incidence days in advance of traditional measures, such as positive tests, hospitalizations, or mortality. We are currently planning additional studies using a broadly representative sample of individuals who will undergo uniform COVID-19 testing to further validate our approach to symptom-based modeling of incidence. These data demonstrate compelling evidence for the potential predictive power of our approach, which will improve as more data are collected to inform the model. Further, our data highlight the potential utility of real-time symptom tracking to help guide allocation of resources for testing and treatment as well as recommendations for lockdown or easement in specific areas.

With additional data collection, we will also apply big data approaches (e.g., machine learning) to identify emerging patterns in dynamic settings of exposure, onset of symptoms, disease trajectory, and clinical outcomes. Our launch of the app within several large epidemiology cohorts that have previously gathered longitudinal data on lifestyle, diet and health factors, and genetic information will allow investigation of a much broader range of putative risk factors for COVID-19 outcomes. With additional follow-up, we will also be positioned to investigate long-term effects of COVID-19, including mental health, disability, mortality, and financial outcomes. Mobile technology can also supplement recently launched clinical trials or biobanking protocols already embedded within clinical settings. In collaboration with the Stand Up to Cancer foundation, we have also developed a strategy to track information among individuals living with cancer, including those enrolled in clinical trials. At the Massachusetts General Hospital and Brigham and Women's Hospital, we are deploying the tool within several clinical studies, centralized biobanking efforts, and health care worker surveillance programs. Health care workers are particularly vulnerable to COVID-19's effects beyond infection, including work hazards from PPE shortages, emotional stress, and absenteeism. Real-time data generation focused within these populations will be critical to optimally allocate resources to protect our health care workforce and assess its efficacy.

Even so, our approach has limitations. We recognize that a smartphone application does not represent a random sampling of the population. However, this is an inherent limitation of any epidemiologic study that relies on voluntary participation. Our approach has the benefit of allowing rapid deployment across a large cross section of the population during a major public health crisis. With time and continued use, the large number of participants will include a sufficient quantity of users within key subgroups such that we can adjust our methodology for potential sources of confounding. By engaging cohorts with underrepresented populations, such as the BWHS in the United States, we also hope to leverage existing investigator-participant relationships to encourage enrollment of individuals from populations that have traditionally been challenging to recruit. Moreover, by encouraging longitudinal, prospective data collection, we can capture associations based on within-person variation over time, a notable advantage over repeated cross-sectional surveys that introduce considerable between-person variation. In the near future, we hope to release our app as fair-use open source software to facilitate translation and development in other regions. We have begun working with colleagues in Canada, Australia, and Sweden to implement this tool within their countries. We have also developed a practical toolkit to assist clinical researchers with local institutional review board and regulatory approval to facilitate deployment within research studies (www.monganinstitute.org/cope-consortium). This toolkit includes full details of the mobile app's questions, consent documents, privacy policies, and terms of use. With broader implementation, data generated from the COVID Symptom Study app are increasingly being linked to the public health response within the National Health Service (NHS) in the United Kingdom. The app is endorsed by the Welsh government, NHS Wales, the Scottish government, and NHS Scotland, and our scientific team updates the U.K. chief scientific officer daily. We are working to develop a similar approach in the United States. However, the lack of a national health care system has required a strategy focused on engaging local public health leaders. For example, we have partnered with the University of Texas School of Public Health to conduct statewide surveillance to support public health decision-making, especially as the Texas state government begins softening mitigation strategies.

Our approach demonstrates a proof of concept for rapid repurposing of existing data collection methods to implement scalable real-time collection of population-level data during a fast-moving global health crisis. We call on our colleagues to work with us so that we may deploy all of the tools at our disposal to address this unprecedented public health challenge.

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distinct mobile application that was supported by Zoe Global Ltd. The other authors have no conflicts of interest to declare. **Data and materials availability:** This manuscript was previously made available as a preprint on medRxiv.org (*19*). Code used to generate maps is published and available in Zenodo (*20*). Data collected in the app are being shared with other health researchers through the NHS-funded Health Data Research UK (HDRUK)/SAIL Consortium, housed in the UK Secure e-Research Platform (UKSeRP) in Swansea, Wales. Anonymized data are available to be shared with bona fide researchers through HDRUK according to their protocols in the public interest. See https://healthdatagateway.org/detail/9b604483-9cdc-41b2-b82c-14ee3dd705f6. U.S. investigators are encouraged to coordinate data requests through the COPE Consortium (www.monganinstitute.org/cope-consortium). Data updates can be found on https://covid.joinzoe.com. For aggregate deidentified "snapshot" datasets used for analyses provided here, please request time-stamped datasets by email to predict@mgh.harvard.edu for data files, "COVIDSymptomTrackerData_03292020" (Figs. 2 and 3), or "COVIDSymptomTrackerData_04/21/20" (Fig. 4). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/. This license does not apply to figures/photos/artwork or other content included in the article that is credited to a third party; obtain authorization from the rights holder before using such material.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/368/6497/1362/suppl/DC1
Figs. S1 to S9
COPE Consortium Members
MDAR Reproducibility Checklist

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MEASLES

Measles virus and rinderpest virus divergence dated to the sixth century BCE

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Many infectious diseases are thought to have emerged in humans after the Neolithic revolution. Although it is broadly accepted that this also applies to measles, the exact date of emergence for this disease is controversial. We sequenced the genome of a 1912 measles virus and used selection-aware molecular clock modeling to determine the divergence date of measles virus and rinderpest virus. This divergence date represents the earliest possible date for the establishment of measles in human populations. Our analyses show that the measles virus potentially arose as early as the sixth century BCE, possibly coinciding with the rise of large cities.

Measles is a highly contagious viral disease that presents with rash, fever, and respiratory symptoms. Before a live-attenuated vaccine was developed in the 1960s, the disease affected the vast majority of children (*1*, *2*). Global vaccination campaigns resulted in a marked reduction of measles transmission and fatal cases, and the World Health Organization (WHO) has proclaimed an elimination goal. However, the disease still caused an estimated 110,000 deaths in 2017 (*3*), and incidence has recently been on the rise (*4*). Measles is caused by *Measles morbillivirus* (MeV), a negative-sense single-stranded RNA virus from the family *Paramyxoviridae* (Order: *Mononegavirales*). MeV is an exclusively human pathogen whose closest relative was the now eradicated *Rinderpest morbillivirus* (RPV), a devastating cattle pathogen (*5*). It is generally accepted that measles emergence resulted from a spillover from cattle to humans, although the directionality of this cross-species transmission event has never been formally established (supplementary text S1) (*6*).

It is unclear when measles first became endemic in human populations, but assuming an origin in cattle, the earliest possible date of MeV emergence is defined by the MeV-RPV divergence time. Several studies have provided estimates for this date using molecular-clock analyses (*7*–*10*), with the most reliable (and oldest) estimate falling at the end of the ninth century CE {mean, 899 CE [95% highest posterior density (HPD) interval, 597 to 1144 CE]} (*8*). We reassessed the MeV-RPV divergence time using advanced, selection-aware Bayesian molecular-clock modeling (*11*) on a dataset of heterochronous MeV genomes, including the oldest human RNA virus genome sequenced to date, and show that a considerably earlier emergence can no longer be excluded.

Our reexamination was prompted by the broadly accepted view that molecular dating based on tip date calibration—the method used in previous efforts to estimate the timing of MeV-RPV divergence—underestimates deep divergence times (*8*). Rapid short-term substitution rates captured with tip date calibration often cannot be applied over long evolutionary time scales because of the effects of long-term purifying selection and substitution saturation. This causes a discrepancy between short- and long-term substitution rates, which is referred to as the time-dependent rate phenomenon (*12*, *13*). Because measurement time scales matter, a first step to arrive at accurate estimates is to maximize the time depth of tip date calibration—for example, through the use of ancient viral sequences (*14*, *15*).

RNA tends to be much less stable in the environment than DNA, making the recovery of MeV genetic material from archaeological remains unlikely (*16*). Pathology collections represent a more realistic source of MeV sequences that predate the oldest MeV genome: the genome of the Edmonston strain that was isolated in 1954 and attenuated to become the first measles vaccine. We examined a collection of lung specimens gathered by Rudolf Virchow and his successors between the 1870s and 1930s and preserved by the Berlin Museum of Medical History at the Charité (Berlin, Germany) and identified a 1912 case diagnosed with fatal measles-related bronchopneumonia (Fig. 1, fig. S1, and supplementary text S2 and S3). To retrieve MeV genetic material from this specimen, we first heat-treated 200 mg of the formalin-fixed lung tissue to reverse macromolecule cross-links induced by formalin and subsequently performed nucleic acid extraction (*17*). After deoxyribonuclease treatment and ribosomal RNA depletion, we built high-throughput sequencing libraries and shotgun sequenced them on Illumina platforms. We generated 27,328,219 high-quality reads, of which 0.46% were mapped to a MeV genome. Median insert size varied between 95 and 136 nucleotides (nt), and little damage was observed, suggesting good preservation of RNA molecules (fig. S2, table S1, and supplementary text S4). The resulting 10,960 unique MeV reads allowed us to reconstruct an almost complete 1912 MeV genome: 15,257 of the 15,894 nt

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Fig. 1. Formalin-fixed lung specimen collected in 1912 in Berlin. Specimen is from a 2-year-old girl diagnosed with measles-related bronchopneumonia (museum object ID: BMM 655/1912).

in the MeV strain Edmonston (AF266288) were covered by at least three unique reads (11,988 nt by at least 20 reads; mean coverage 54x).

In addition to the 1912 genome and the 1954 Edmonston genome, only two genomes have been determined from MeV isolated before 1990 (Mvi/Lyon.FRA/77, HM562899; and T11wild, AB481087). We therefore searched the strain collection of the German National Reference Laboratory (Robert Koch Institute, Berlin, Germany) for pre-1990 isolates. We found two strains from the pre-vaccine era isolated in 1960 by the National Reference Laboratory of former Czechoslovakia in Prague (MVi/Prague.CZE/60/1 and MVi/Prague.CZE/60/2) (*18*). We performed serial passages of these strains and determined their genome sequences at a mean coverage of 109x and 70x, respectively. The two genomes were nearly identical, differing at only four sites.

We performed Bayesian and maximum likelihood (ML) phylogenetic analyses to investigate the phylogenetic placement of the 1912 and 1960 genomes with respect to 127 available MeV genomes. Tip-dated Bayesian phylogenetic trees placed the 1912 genome as a sister lineage to all modern genomes, whereas the two genomes from 1960 clustered together with the Edmonston strain (genotype A) (fig. S3). The placement of the 1912 genome in the dated-tip tree was consistent with its placement in a non-clock ML tree reconstruction

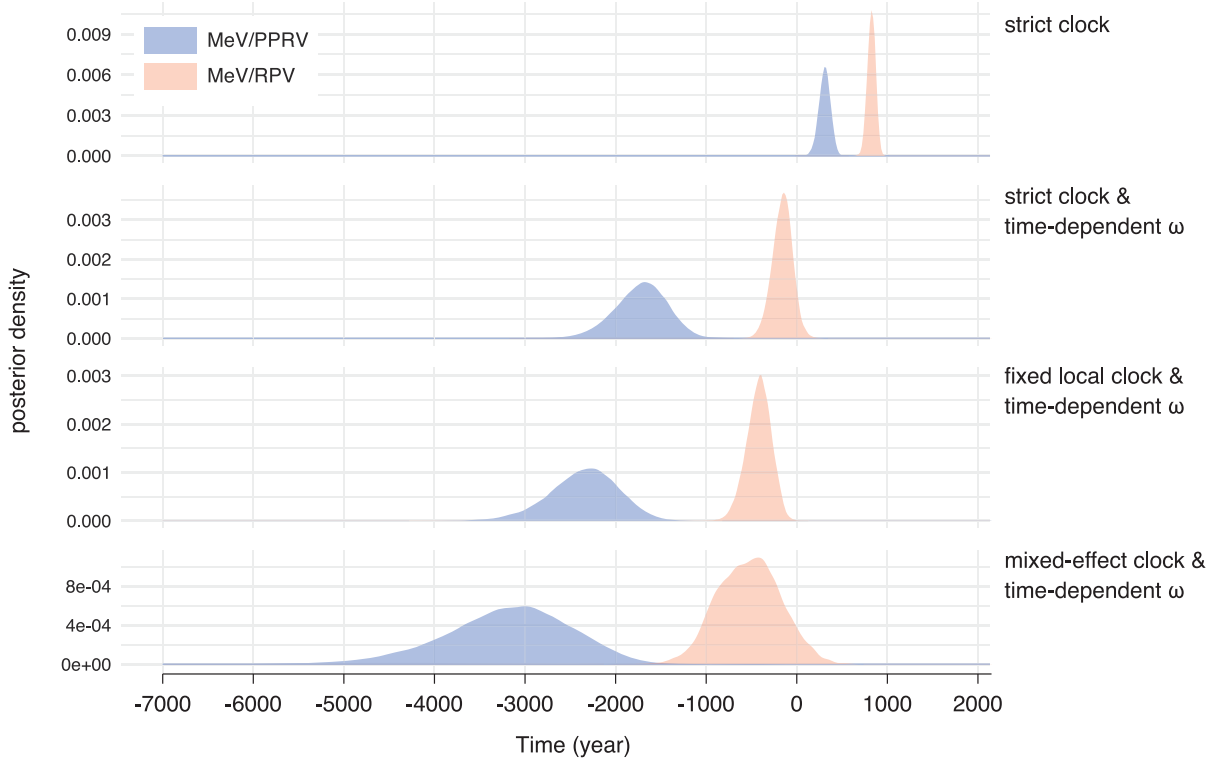

Fig. 2. Divergence time estimates for MeV and RPV (red) and for MeV-RPV and PPRV (blue) under increasingly complex evolutionary models. Estimates for parameters of interest (posterior mean and 95% highest posterior density interval) under each model are provided in table S5.

and with the rooting of a dated-tip tree excluding the 1912 genome (fig. S4 A and B). The relatedness of the 1912 and 1960 genomes to now extinct MeV lineages is in line with a marked reduction of MeV genetic diversity during the 20th century as a product of massive vaccination efforts.

Having extended the depth of MeV tip date calibration, we subsequently focused our attention on estimating the timing of MeV-RPV divergence. We assembled a dataset of 51 genomes comprising MeV (including one of the 1960 genomes and the 1912 genome), RPV, and Peste des petits ruminants virus (PPRV; the closest relative to MeV-RPV) sequences, ensuring that they represented the known genetic diversity of these viruses (table S2). Before inferring a time-scaled evolutionary history for this dataset by using a Bayesian phylogenetic framework, we assessed its temporal signal and tested it for substitution saturation. We confirmed a strong temporal signal (fig. S5 and table S3) and did not identify strong substitution saturation (table S4). We constructed a series of increasingly complex evolutionary models to accommodate various sources of rate heterogeneity. Models ranged from a standard codon substitution model with a strict molecular clock assumption to a codon substitution model with time-varying selection combined with a clade-specific rate for PPRV and additional branch-specific random effects on the substitution rate. Adequately accommodating different sources of rate heterogeneity is known to provide a better correction for multiple hits in genetic distance estimation, and the potential of codon substitution modeling in recovering deep viral divergence has been demonstrated specifically (*8*). This was reflected in the increasingly older estimates of MeV-RPV and PPRV-MeV-RPV divergence times and wider credible intervals for increasingly complex models (Fig. 2 and table S5). Parameter estimates of the substitution and clock models also provided evidence for a significant contribution of these different sources of rate heterogeneity to model fit improvement (table S5). We found a significantly negative coefficient for the time-dependent nonsynonymous/synonymous substitution rate ratio (ω) (*11*), indicating strong long-term purifying selection; a significantly positive coefficient for the fixed effect on the PPRV rate, indicating a faster evolutionary rate in this clade (as suggested by temporal signal analyses, fig. S5); and significant additional unexplained variation, as modeled by the random effects (table S5). Our most complex model therefore provided the best description of the evolutionary process and pushed back the divergence date of MeV and RPV, with a mean estimate at 528 BCE (95% HPD interval, 1174 BCE to 165 CE) (Fig. 3). These estimates were robust to (i) including or excluding the 1912 genome in the analyses (table S6), (ii) using a more conservative consensus genome for the 1912 sample (table S7), (iii) the prior specification on the age of the RPV genome or the inclusion of an additional RPV genome (table S7), and (iv) the coalescent prior specification (table S7). A comparison of the four models in their ability to recover the age of the 1912 genome indicated that the most complex model yielded the best estimate [1929 CE (95% HPD interval, 1889 to 1961 CE)] (fig. S6).

The MeV-RPV divergence time provides the earliest possible date for measles emergence in humans, which is now compatible with the emergence of this disease more than 2500 years ago. It seems plausible that the divergence of these lineages was closely followed by the cattle-to-human host jump and subsequent evolution into two distinct pathogens. However, the spillover could have occurred at any time between the MeV-RPV divergence and the time to the most recent common ancestor of all MeV known to infect humans [1880 CE (95% HPD interval, 1865 to 1893 CE)]. This raises the question of whether other sources of information can narrow down this time frame and agree with an earlier timing of measles emergence.

The earliest clear clinical description of measles is often attributed to the Persian physician Rhazes, writing in the 10th century CE (*19*). But Rhazes was extremely familiar with all available medical literature at his time and made use of earlier sources. Indian medical texts possibly describe measles several centuries before Rhazes (*20*). Although clear descriptions of measles are missing in the Hippocratic corpus

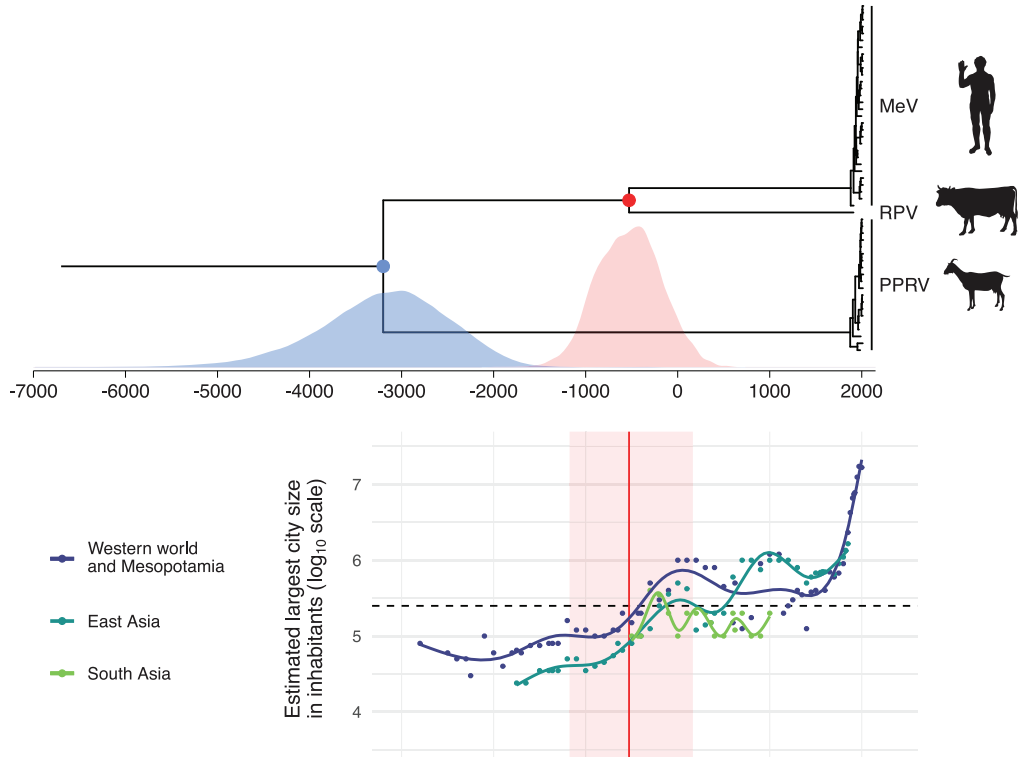

Fig. 3. Time-measured evolutionary history for MeV, RPV, and PPRV and largest city size over time in three well-studied regions of the world. (**Top**) Maximum clade credibility tree summarized from a Bayesian time-measured inference by using tip-dating and accounting for long-term purifying selection. The red and blue points represent the mean estimates for the divergence times between MeV and RPV then MeV-RPV and PPRV, respectively; the corresponding divergence date estimates are depicted below as marginal posterior distributions. (**Bottom**) The estimated size (log10 scale) of the largest city in the western world including Mesopotamia (dark blue), East Asia (dark green), and South Asia (green) over time. The red vertical line indicates the mean divergence time estimate between MeV and RPV, and the red area indicates its 95% highest posterior density interval. The dashed horizontal line represents the classical threshold for MeV maintenance in a population (250,000 individuals). Dots show data points according to Morris (*34*) and Inoue *et al*. (*26*). Each line represents the fit of a generalized additive model with a cubic spline smoothing function.

and the Greek medical tradition (at least through the prolific second-century writer Galen), such absence alone cannot be decisive. Retrospective diagnosis from premodern medical texts is notoriously fraught, especially for diseases such as measles, whose symptoms were easily confused with a variety of other conditions. Measles differential diagnosis remained a challenge well into more recent times (*21*). Therefore, any number of the large-scale "pestilences" described in ancient sources from Europe or China could reflect MeV outbreaks.

An ancient origin of measles seems all the more plausible in the light of demographic changes that are compatible with our understanding of (contemporary) MeV epidemiology. Populations large enough to support continuous MeV transmission—larger than the MeV critical community size (CCS) of 250,000 to 500,000 individuals (*22*–*24*)—could not exist in Neolithic, Bronze Age, and early Iron Age settlements, which lacked both economic and political means to allow such numbers. Even if connectivity between such settlements may have created a larger pool of susceptible individuals, epidemiologists have held that given the speed with which measles epidemics occur and the efficacy of acquired immunity, MeV could not have become endemic in urban populations below the CCS (*25*). In the late first millennium BCE, technologies (both economic and political) crossed a threshold, promoting an upsurge in population sizes in Eurasia and South and East Asia. Although considerable uncertainty exists around population size estimates derived from ancient documents (for example, literary observations, travelers' reports, censuses, or references to the amount of food distributed in a city) or archaeological proxies (such as the size of city walls or a built-up area of settlement), there is broad agreement that a number of settlements in North Africa, India, China, Europe, and the Near East began to surpass the CCS for MeV by around 300 BCE, presumably for the first time in human history (Fig. 3) (*26*). From this period onward, there were consistently urban populations above the CCS for MeV.

On the basis of these considerations, our substantially older MeV-RPV divergence estimate provides grounds for sketching a new model of MeV's evolutionary history. Under this scenario, a bovine virus, the common ancestor of modern strains of RPV and MeV, circulated in large populations of cattle (and possibly wild ungulates) since its divergence from PPRV around the fourth millennium BCE [3199 BCE (95% HPD interval, 4632 to 1900 BCE)] (Fig. 3). As a fast-evolving RNA virus, it may have produced variants that were able to cross the species barrier on several occasions, but small human populations could only serve as dead-end hosts. Then, almost as soon as contiguous settlements reached sufficient sizes to maintain the virus' continuous transmission (Fig. 3), it emerged as a human pathogen, the progenitor of modern-day MeV. It has been suggested that numerous concurrent human-bovine epidemics in the early medieval period (here, 6th to 10th centuries CE) were caused by an immediate ancestor of MeV and RPV that was pathogenic to both cattle and humans (*27*). The new MeV-RPV divergence date allows for the same inference to be made for earlier concurrent human-bovine mortality events well attested in, for example, Roman sources from the fifth century BCE on (*28*). During the following centuries, introduction of MeV into naive human populations and/or flare-ups of the disease might have caused some ancient epidemics whose etiology remains uncertain.

Although our findings shed new light on the origin of measles, formally proving that the virus emerged soon after its divergence from RPV would require archaeological genomic evidence. Most studies on ancient viruses have thus far focused on viruses with a double-stranded DNA genome (*14*, *29*–*32*). However, genetic material of parvovirus B19 was also detected in early Neolithic skeletal remains, despite the relatively unstable nature of its single-stranded DNA genome (*15*). It remains to be determined whether viral RNA recovery from such ancient specimens is feasible. Recently, RNA was extracted from the remains of a 14,300-year-old Pleistocene canid preserved in permafrost (*33*). Although the majority of RNA fragments were extremely short (<30 nt), the authenticity of the sequences could be validated (*33*). Such advances highlight that it may not be completely impossible for ancient remains to still contain MeV RNA, especially if preserved under favorable circumstances, including natural mummification or preservation in cold environments (*16*). While awaiting such direct evidence, we believe that the proposed model of MeV evolution constitutes a compelling working hypothesis.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text S1 to S4
Figs. S1 to S6
Tables S1 to S7
References (*36*–*83*)

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IMMUNOMETABOLISM

T cells with dysfunctional mitochondria induce multimorbidity and premature senescence

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The effect of immunometabolism on age-associated diseases remains uncertain. In this work, we show that T cells with dysfunctional mitochondria owing to mitochondrial transcription factor A (TFAM) deficiency act as accelerators of senescence. In mice, these cells instigate multiple aging-related features, including metabolic, cognitive, physical, and cardiovascular alterations, which together result in premature death. T cell metabolic failure induces the accumulation of circulating cytokines, which resembles the chronic inflammation that is characteristic of aging ("inflammaging"). This cytokine storm itself acts as a systemic inducer of senescence. Blocking tumor necrosis factor–α signaling or preventing senescence with nicotinamide adenine dinucleotide precursors partially rescues premature aging in mice with *Tfam*-deficient T cells. Thus, T cells can regulate organismal fitness and life span, which highlights the importance of tight immunometabolic control in both aging and the onset of age-associated diseases.

The emerging field of immunometabolism (*1*–*5*) has expanded therapeutic opportunities for the treatment of not only inflammatory and autoimmune disorders (*6*) but also metabolic diseases and cancer (*7*–*10*). However, the utility of targeting immunometabolism to prevent the onset of age-associated diseases and multimorbidity has not been previously addressed.

An age-related decline in mitochondrial function has been observed in various cells and tissues, including T cells (*11*). To investigate the consequences of T cell metabolic decline in healthy aging, we used $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice. *Tfam* is a nuclear gene that encodes mitochondrial transcription factor A (TFAM), which both stabilizes mitochondrial DNA (mtDNA) and initiates mtDNA replication (*12*). In $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice, *Tfam* is depleted in both CD4 and CD8 T lymphocytes (*13*). T cell *Tfam* deficiency reduced the numbers of total circulating CD4 and CD8 T cells (fig. S1A). Lack of *Tfam* resulted in a sharp decrease in T cell mtDNA content (fig. S1B) and the failure to express key electron transport chain components, which reprogrammed T cell metabolism toward glycolysis (*13*) (Fig. 1, A to C). T cells from young (2-month-old) $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice recapitulated features of the mitochondrial dysfunction that appears in aged (22-month-old) wild-type mice (Fig. 1, A to C). This mitochondrial decline was associated with T helper 1 (T_H1) cell skewing, characterized by higher secretion of inflammatory type 1 cytokines interferon-γ (IFN-γ) and tumor necrosis factor–α (TNF-α), and the increased expression of the T_H1 cell master regulator T-bet (Fig. 1, D and E, and fig. S1C). In addition to this proinflammatory phenotype, young (2-month-old) $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice were immunocompromised to a similar extent as old (22-month-old) wild-type mice. We infected $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice with ectromelia virus (ECTV), a highly virulent mouse poxvirus that causes a disease similar to human smallpox, and compared them with young control or old wild-type mice. Infection with ECTV killed old wild-type mice and young $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice within the first 10 days of infection, whereas all young controls were able to survive this acute infection (Fig. 1F). Thus, *Tfam*-deficient T cells recapitulate the metabolic, phenotypic, and functional features of aged T cells. Seven-month-old $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice exhibited premature inflammaging, with circulating levels of the inflammaging-associated cytokines IL-6, IFN-γ, and TNF-α similar to the levels observed in 22-month-old wild-type mice (Fig. 1G and fig. S1, D and E). In humans, inflammaging predicts susceptibility to cardiovascular diseases, neurodegeneration, frailty, and multimorbidity (*14*–*16*). $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice displayed a prematurely aged appearance from the age of 7 months (Fig. 1H), which progressed to anemia, kyphosis, and low body weight (Fig. 1, I to K, and fig. S1F). An additional indicator of aging in $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice was the significant thinning of hypodermal fat (Fig. 1L). Consistent with a premature aging phenotype, metabolic cage experiments revealed that $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice were less active and slower than controls, despite higher energy expenditure (Fig. 1M and fig. S1G). Notably, mean life span in $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice was half that of controls (483 days versus 984 days) (Fig. 1N). Although *Tfam* deletion in regulatory T cells induces lethal autoimmunity (*17*), $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice showed no differences in serum autoantibody levels (fig. S1H).

To evaluate the potential of $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice as a model of age-related multimorbidity, we analyzed muscular, cardiovascular, and cognitive fitness. Histological analyses of $Tfam^{fl/fl}$ $Cd4^{Cre}$ muscle tissue revealed a reduced fiber diameter (Fig. 2A). Imaging analysis of the gastrocnemius muscle after injection of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) revealed significantly lower glucose uptake in $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice (Fig. 2B), and lower skeletal muscle strength was confirmed by the grip strength test (Fig. 2C). Moreover, $Tfam^{fl/fl}$ $Cd4^{Cre}$ muscle up-regulated the expression of genes encoding the ubiquitin ligases MuRF-1 and Atrogin-1 as well as the expression of inflammatory markers (Fig. 2D and fig. S2A). $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice displayed a marked loss of gonadal white adipose tissue (gWAT) mass and smaller adipocytes (Fig. 2, E and F). These differences were accompanied by elevated expression levels of the adipose triglyceride lipase in gWAT and elevated circulating levels of nonesterified fatty acids, both of which were indicative of lipolysis induction (Fig. 2, G and H). Thus, the loss of T cell immunometabolic control induces sarcopenia and lipolysis. Additionally, $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice showed evidence of cardiac atrophy (Fig. 2I and fig. S2B). Histological and echocardiographic analyses revealed relative reduction in left ventricular thickness, reduced left ventricular diameter and volume, and smaller cardiomyocyte size (Fig. 2J and fig. S2, C to F). These features were accompanied by a higher heart rate (Fig. 2K) and the induction of cardiac stress markers *Foxo3a* and *Nppa* (fig. S2G). $Tfam^{fl/fl}$ $Cd4^{Cre}$ mice displayed diastolic failure, characterized by reduced cardiac output, as well as elevated normalized lung weight with signs of lung congestion, as shown by computed tomography scan (Fig. 2, L and M, and fig. S2H).

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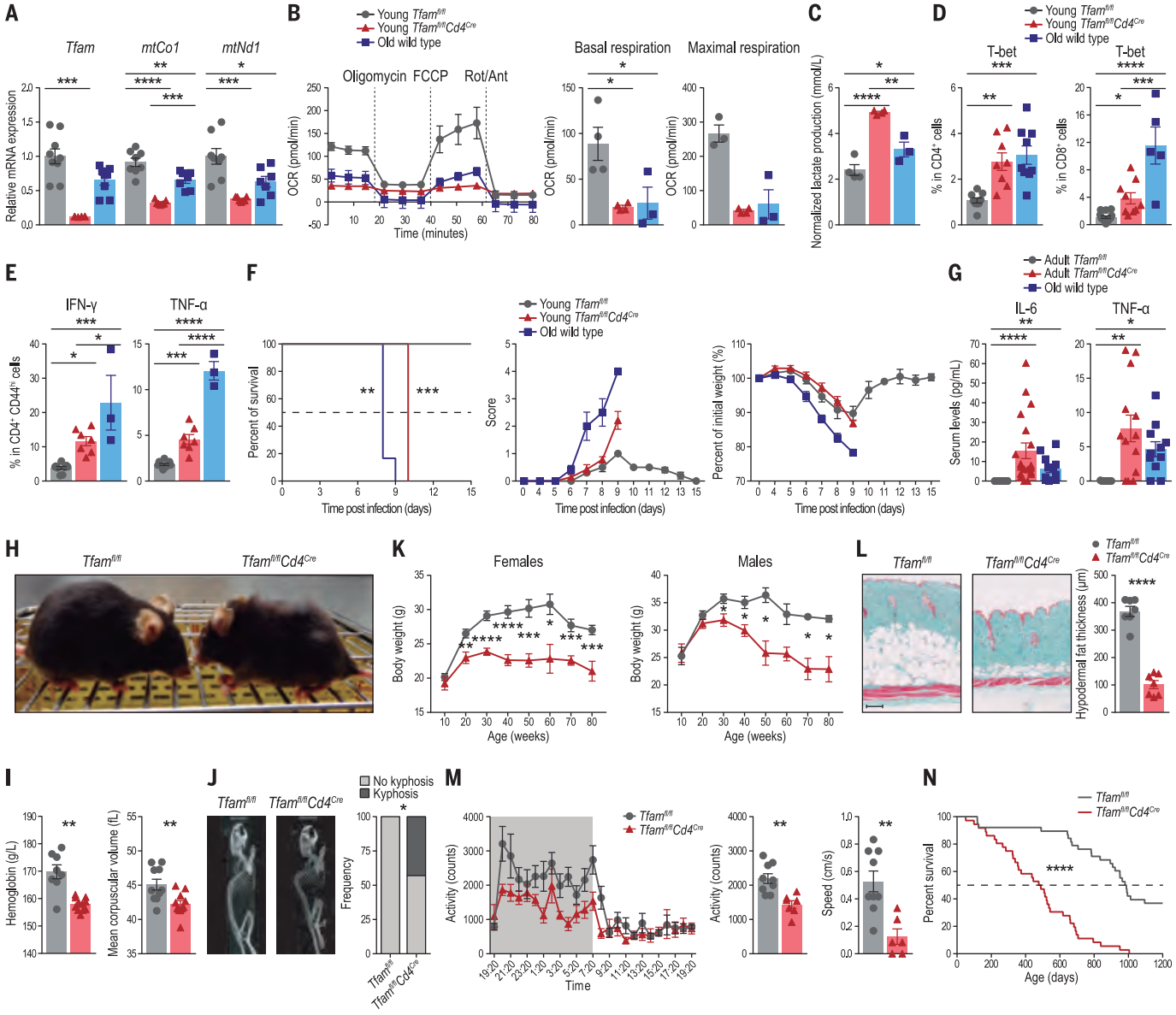

Fig. 1. Mitochondrial dysfunction in T cells causes premature aging. (**A**) *Tfam*, *mtCo1*, and *mtNd1* mRNA levels in peripheral blood CD4$^+$ T cells from young (2-month-old) *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice and old (22-month-old) wild-type (WT) mice (n = 6 to 9 mice per group). (**B**) (Left) Oxygen consumption rates (OCR) in activated CD4$^+$ T cells from *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and old WT animals. Basal respiration (center) and maximal respiratory capacity (right) are shown (n = 3 to 4). FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; Rot, rotenone; Ant, antimycin A. (**C**) Lactate content in the supernatant of activated CD4$^+$ T cells (n = 3 to 4). (**D**) Percentages of CD4$^+$ (left) and CD8$^+$ (right) T cells positive for the T_H1 cell transcription factor T-bet (n = 5 to 14). (**E**) Percentages of CD4$^+$CD44^{hi} T cells staining positive for intracellular IFN-γ and TNF-α (n = 3 to 9). (**F**) Postinfection survival curves (left) for *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and old WT mice inoculated subcutaneously with ECTV [10^3 plaque-forming units (PFU) per mouse]. ECTV-infected mice were monitored daily for clinical signs of illness (center) and change from initial body weight (right). Signs of illness are expressed as means ± SEM using an individual score ranging from 0 for healthy animals to 4 for severely diseased animals (n = 5 to 7). (**G**) Serum levels of inflammatory cytokines IL-6 and TNF-α detected by multiplex in 7-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice and 22-month-old WT mice (n = 10 to 19). (**H**) Representative photograph showing the deteriorated physical appearance of a *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mouse (right) compared with a control littermate (left), both aged 7 months. (**I**) Hematological parameters in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} and *Tfam*$^{fl/fl}$ mice (n = 8 to 11 5-month-old mice). (**J**) Quantification of spine curvature by computed tomography (CT) scans (left) and percentage of mice presenting lordokyphosis (right) in 5-month-old *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 7 to 8). (**K**) Body weight evolution in *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} female (left) and male (right) mice (n = 8 to 20). (**L**) Representative skin sections stained with Masson trichrome (left) and quantification of hypodermal fat thickness (right). At least 10 measurements were performed per animal. The graph shows mean values for n = 7 mice at 7 months of age. Scale bar, 100 μm. (**M**) Activity time course over a 24-hour cycle (left) and mean dark period activity (center) and speed (right) assessed in metabolic cages (n = 6 to 9 7-month-old mice). Bar graphs correspond to the dark (active) period. (**N**) Kaplan-Meier survival curves for *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 36 to 38 mice, including males and females). Dots in all panels represent individual sample data. Data are presented as means ± SEM. Statistical analysis was by one-way analysis of variance (ANOVA) with post hoc Tukey's correction [(A), *mtCo1* and *mtNd1*; (C), (D), and (G), TNF-α]; Kruskal-Wallis H test with post hoc Dunn's correction [(A), *Tfam*; (B) and (G), IL-6]; unpaired Student's t test [(I), MCV; (J), (K), and (M)]; or unpaired Welch's t test [(L) and (I), hemoglobin]. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Survival curve data were analyzed by log-rank (Mantel-Cox test) [(F) and (N)]. For the ECTV experiment, data correspond to one of two representative experiments.

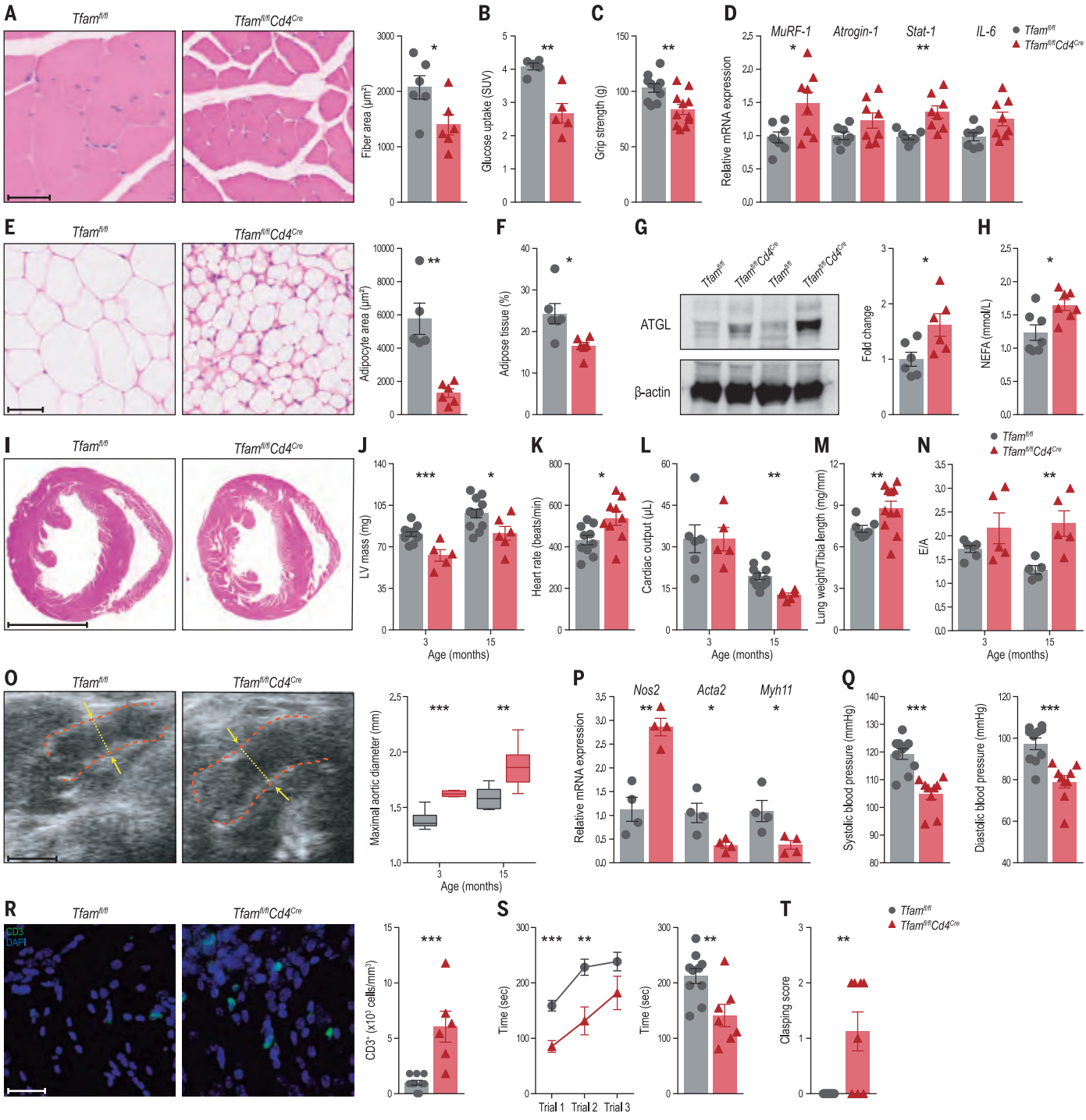

Fig. 2. *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice develop age-associated multimorbidity. (**A**) Representative hematoxylin and eosin (H&E)–stained sections of the gastrocnemius muscle (left; scale bar, 50 μm) and quantification of myofiber cross-sectional area (right). At least 10 measurements were performed per animal. The graph shows mean fiber area for n = 6 animals per group (7-month-old mice). (**B**) In vivo positron emission tomography and CT analysis of skeletal muscle glucose uptake. Data are means ± SEM of ^{18}F-FDG activity (n = 5 4-month-old mice). SUV, standard uptake value. (**C**) Forelimb grip strength analysis (n = 10 to 11 7-month-old mice). (**D**) Relative mRNA levels of genes related to muscle proteolysis [*Murf1* and Atrogin-1 (*Fbxo32*)] and inflammation (*Stat1* and *Il6*) (n = 7 to 8 7-month-old mice). (**E**) Representative H&E-stained sections of gWAT from *Tfam*$^{fl/fl}$ *Cd4*^{Cre} and *Tfam*$^{fl/fl}$ mice (left; scale bar, 50 μm). The graph (right) shows mean estimated adipocyte surface area. Ten measurements were performed per animal (n = 5 to 6 animals, 7-month-old mice). (**F**) Percentage of adipose tissue determined by quantitative magnetic resonance imaging in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} and *Tfam*$^{fl/fl}$ mice (n = 6 7-month-old mice). (**G**) Immunoblot (left) and densitometry analysis (right) of adipose triglyceride lipase (ATGL) protein expression in gWAT isolated from *Tfam*$^{fl/fl}$ *CD4*^{Cre} mice and control littermates (n = 6 7-month-old mice). (**H**) Plasma nonesterified fatty acids (NEFA) (n = 7 4-month-old mice). (**I**) Representative H&E-stained heart sections from 15-month-old *Tfam*$^{fl/fl}$ *Cd4*^{Cre} and *Tfam*$^{fl/fl}$ mice. Scale bar, 2.5 mm. (continued on next page)

(**J**) Echocardiography measurements of left ventricular (LV) mass in 3- and 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 5 to 12). (**K**) Heart rate in 3-month-old *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice and control littermates (n = 9 to 10). (**L**) Cardiac output in 3- and 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 5 to 11). (**M**) Lung weight normalized to tibia length in *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 7 to 11 7-month-old mice). (**N**) Mitral flow pattern on echocardiography in 3- and 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 5 to 6). E/A, E wave/A wave ratio. (**O**) Representative ultrasound images depicting maximal ascending aorta diameters in 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (left; scale bar, 1 mm) and quantification of maximal aortic diameter in 3- and 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (right) (n = 6 to 8). Maximal aortic diameter is presented in box-and-whisker plots showing the median, maximal, and minimal values and 75th and 25th percentiles. (**P**) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of *Nos2*, *Acta2*, and *Myh11* mRNA expression levels in aortic samples from 7-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 4). (**Q**) Systolic and diastolic blood pressure in 3-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 9 to 10). (**R**) Representative CD3-stained brain (fornix) sections from 15-month-old *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (left; scale bar, 25 μm) and quantification of CD3 positive cell density (right) (n = 6 to 10). DAPI, 4′,6-diamidino-2-phenylindole. (**S**) Rotarod test performance by *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice, expressed as the mean time spent on the rotating rod in each of three trials (left) and in all trials combined (right) (n = 7 to 10 12-month-old mice). (**T**) Maximum clasping score per 30-s test (n = 8 to 9 12-month-old mice). Dots in all panels represent individual sample data. Data are presented as means ± SEM. Statistical analysis was by unpaired Student's t test [(A) to (C) and (D), *Murf1*, *Fbxo32*, and *Il6*; (F) to (L), (N), and (O), 15 months; (P) and (Q), diastolic pressure; and (S)]; unpaired Welch's t test [(D), *Stat1*; (M), (N), and (O), 3 months]; or nonparametric Mann-Whitney U test [(Q), systolic pressure; (R) and (T)]. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

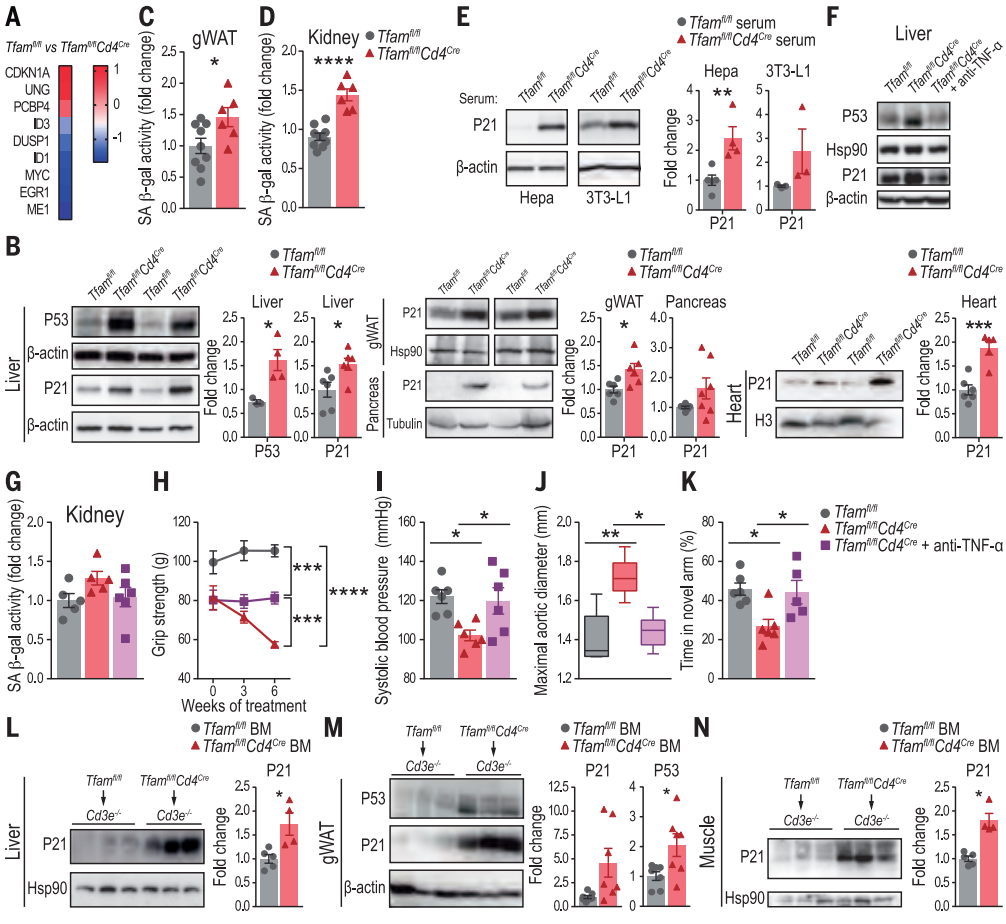

Fig. 3. Inflammaging induces senescence in distal tissues of *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice. (**A**) Heatmap of senescence gene expression changes comparing livers from *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice with those from control littermates. (**B**) Representative immunoblot (left) and quantification (right) of p21 and p53 protein expression in the liver, heart, gWAT, and pancreas from *Tfam*$^{fl/fl}$ *Cd4*^{Cre} and *Tfam*$^{fl/fl}$ mice. Loading controls were β-actin, H3, Hsp90, or α-tubulin (n = 4 to 7 7-month-old mice). (**C**) Quantitative measurements of senescence-associated β-galactosidase (SA β-gal) activity in gWAT lysates by colorimetric assay (n = 6 to 9 9-month-old mice). (**D**) Quantitative measurements of SA β-gal activity in kidney lysates by colorimetric assay (n = 6 to 9 12-month-old mice). (**E**) Immunoblot (left) and densitometry analysis (right) of p21 expression in immortalized mouse hepatocytes (Hepa) and 3T3-L1 cells cultured for 7 days in the presence of *Tfam*$^{fl/fl}$ or *Tfam*$^{fl/fl}$ *Cd4*^{Cre} serum from 7-month-old mice. β-actin was used as a loading control (n = 4 to 6). Blots are representative of three (3T3-L1) or four (hepatocytes) experiments using pooled sera from three animals. (**F**) Representative immunoblot analysis of senescence markers in the liver from control *Tfam*$^{fl/fl}$ and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice with or without treatment with anti–TNF-α (etanercept) (n = 6, 10 weeks of treatment starting from 4 months of age). (**G**) SA β-gal activity measured by a colorimetric assay in kidney lysates (n = 6, 10 weeks of treatment). (**H**) Time course of forelimb strength during anti–TNF-α treatment. (**I**) Systolic blood pressure after 7 weeks of anti–TNF-α treatment (n = 6). (**J**) Maximal ascending aorta diameter in response to anti–TNF-α treatment (n = 6, 8 weeks of treatment). Maximal aortic diameter is presented in box-and-whisker plots showing maximal and minimal values and 75th and 25th percentiles. (**K**) Y-maze analysis in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice treated with anti–TNF-α and corresponding controls (n = 6, 8 weeks of treatment). (**L** to **N**) Immunoblot (left) and densitometry analysis (right) of p21 or p53 expression in liver (L), gWAT (M), and tibialis muscle (N) from *Cd3e*$^{-/-}$ mice 16 weeks after reconstitution with BM from *Tfam*$^{fl/fl}$ *Cd4*^{Cre} or *Tfam*$^{fl/fl}$ mice (n = 4 to 6 per group). Dots in all panels represent individual sample data. Data are presented as means ± SEM. Statistical analysis was by one-way ANOVA with post hoc Tukey's correction [(G), (I), and (K)]; Kruskal-Wallis test with post hoc Dunn's correction (J); two-way ANOVA with post hoc Tukey's correction (H); unpaired Student's t test [(B), (C), (D), (E), (L), and (M)]; or nonparametric Mann-Whitney U test (N). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

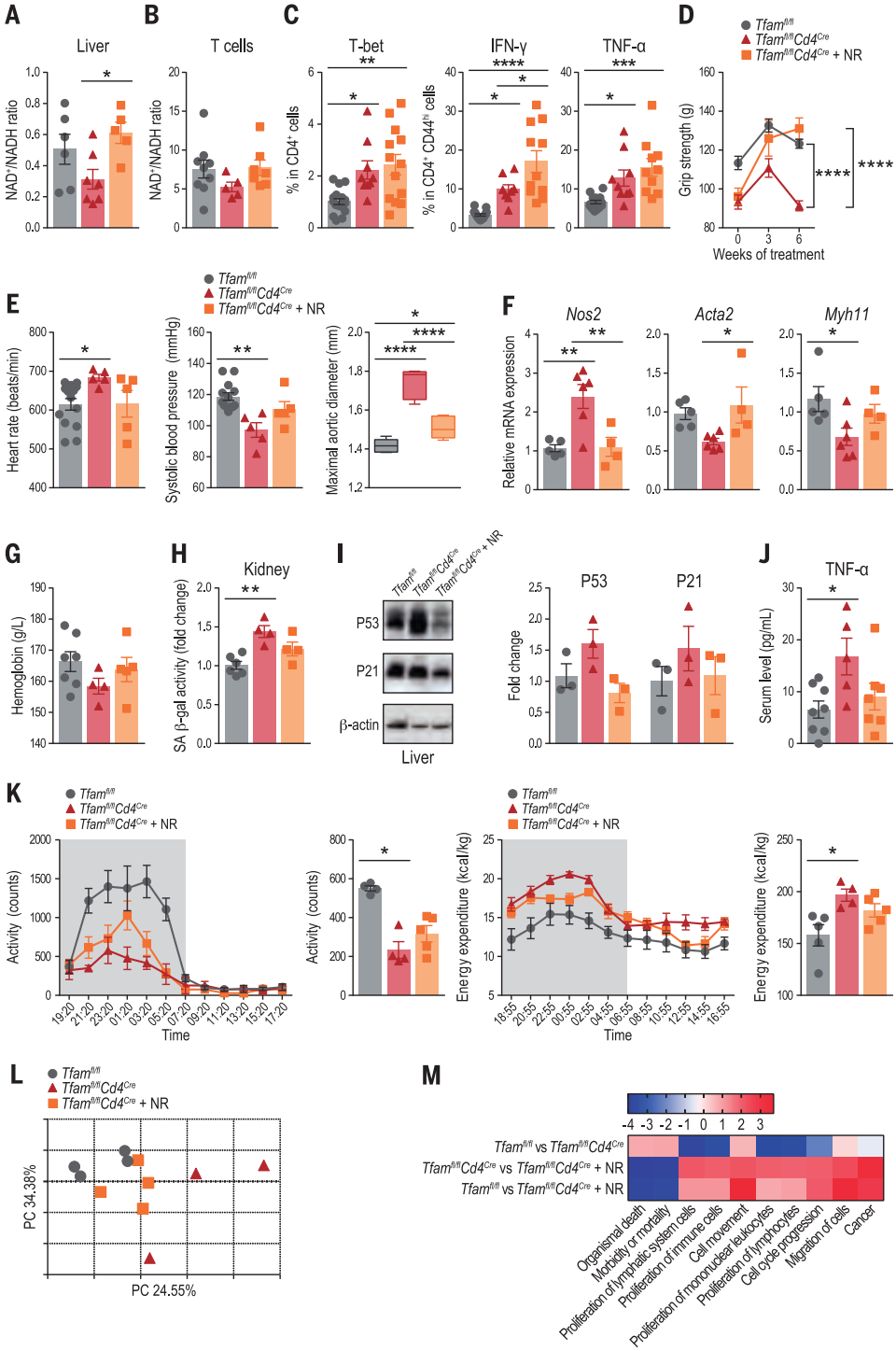

Fig. 4. NR treatment rescues the multimorbidity syndrome. (**A** and **B**) NAD^+/NADH ratio in liver lysates (A) and in isolated $CD4^+$ T cells (B) from untreated *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and NR-treated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 5 to 9 per group, 10-week treatment starting from 4 months of age). (**C**) Flow cytometry analysis of intracellular T-bet (left), IFN-γ (center), and TNF-α (right) in splenic $CD4^+$ T cells (n = 8 to 17 mice). (**D**) Effect of NR treatment on the evolution of forelimb strength (n = 5 to 11). (**E**) Heart rate (left), systolic blood pressure (center), and ascending aorta (AsAo) maximum diameter (right) obtained from ultrasound images after 8 weeks of NR treatment. Maximal aortic diameter is presented in box-and-whisker plots showing the median and the 25th and 75th percentiles (n = 5 to 14). (**F**) RT-qPCR analysis of *Nos2*, *Acta2*, and *Myh11* mRNA expression in the aorta from untreated *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and NR-treated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 4 to 6). (**G**) Blood hemoglobin levels (n = 4 to 7). (**H**) SA β-gal activity measured by colorimetric assay in kidney lysates (n = 4 to 6). (**I**) Immunoblot (left) and densitometry analysis (right) of p21 and p53 expression in liver lysates from untreated *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice treated with NR for 10 weeks (n = 3 to 4). β-actin was used as a loading control. (**J**) Circulating TNF-α determined by multiplex analysis in serum from untreated *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and NR-treated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (n = 5 to 8). (**K**) Activity (left) and energy expenditure (right) monitored over a 24-hour cycle in metabolic cages (n = 4 to 5). (**L**) Principal components (PC) analysis of transcriptomics in liver samples from untreated *Tfam*$^{fl/fl}$, *Tfam*$^{fl/fl}$ *Cd4*^{Cre}, and *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice treated with NR for 10 weeks (n = 3 to 4). (**M**) IPA heatmap showing transcriptionally altered cellular pathways. Dots in all panels represent individual sample data. Data are presented as means ± SEM. Statistical analysis was by one-way ANOVA with post hoc Tukey's correction [(A) to (C) and (E), maximal aortic diameter; (G), (H), and (I), p21; (J) and (K), energy expenditure]; Kruskal-Wallis H test with post hoc Dunn's correction [(E), heart rate and systolic blood pressure; (F) and (I), p53; and (K), activity]; or two-way ANOVA with correction post hoc Tukey's correction (D). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Diastolic dysfunction was also evident from left ventricular relaxation defects detected in the mitral flow pattern (Fig. 2N). *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice developed an age-dependent aortic dilation (Fig. 2O), which is correlated with increased mRNA expression of inducible nitric oxide synthase (*Nos2*), decreased mRNA expression of the contractile markers smooth muscle actin (*Acta2*) and smooth muscle–specific myosin heavy chain (*Myh11*), and decreased blood pressure (Fig. 2, P and Q). Consistent with these findings, 77% of *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice exhibited aortic regurgitation compared with 16% of controls. Histological analysis of the aortas allowed us to identify aortic dissections associated with inflammatory foci in 50% of *Tfam*$^{fl/fl}$ *Cd4*^{Cre} animals (fig. S2I). Thus, *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice appear to develop cardiac atrophy, overt heart failure, and severe cardiovascular alterations, all of which can precipitate death. *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice also showed signs of neurological disability, including an influx of T cells in the fornix region of the brain and defects in motor coordination in both the rotarod and the tail suspension tests (Fig. 2, R to T). Together, these data support a role for T cells beyond host defense (*18*, *19*) and indicate that the metabolic fitness of T cells is critical for organismal homeostasis.

To verify whether this multimorbidity phenotype was caused by a mitochondrial failure in T cells, we used an alternative approach to delete *Tfam* in T cells. We generated *Tfam*$^{fl/fl}$ *Lck*^{Cre} mice, in which Cre recombinase is expressed under the control of the lymphocyte protein tyrosine kinase (*Lck*) promoter. *Tfam*$^{fl/fl}$ *Lck*^{Cre} mice presented comparable metabolic and functional alterations in T cells and similar premature age-associated multimorbidity to those observed in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (fig. S3). To identify the molecular mechanism by which T cell metabolic failure drives organismal frailty and multimorbidity, we performed liver transcriptomics. *Tfam*$^{fl/fl}$ *Cd4*^{Cre} livers showed significant up-regulation of genes associated with senescence (fig. S4A). One of the most up-regulated of these transcripts was *Cdkn1a*, which encodes the cyclin inhibitor p21 (Q value = 0.003) (Fig. 3A). *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice showed elevated protein levels of senescence markers p21$^{Waf/Cip1}$ and p53 in the liver. p21 was also elevated in the heart, gWAT, and pancreas (Fig. 3B). Additionally, the activity of senescence-associated β-galactosidase was higher in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} gWATs and kidneys than in those of controls (Fig. 3, C and D, and fig. S4, B and C). Analysis of *Tfam*$^{fl/fl}$ *Lck*^{Cre} mice confirmed senescence induction in various tissues (fig. S3M).

Because the in vitro incubation of cancer cells with the type 1 cytokines has been shown to induce senescence (*20*), we hypothesized that the type 1 cytokines present in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice drive systemic senescence. Incubation of mouse cells with *Tfam*$^{fl/fl}$ *Cd4*^{Cre} serum or TNF-α was sufficient to increase p21$^{Waf/Cip1}$ levels in hepatocytes and preadipocytes, which supports the argument that inflammatory mediators induce senescence and premature aging (Fig. 3E and fig. S4D). To dissect the contribution of TNF-α to the multimorbidity phenotype, we treated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice with the TNF-α inhibitor etanercept. Blocking TNF-α prevented systemic senescence (Fig. 3, F and G) and muscle, cardiovascular, and cognitive alterations (Fig. 3, H to K). Bone marrow (BM) transplantation from *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice into T cell–deficient (*Cd3e*$^{-/-}$) mice (fig. S5, A and B) recapitulated the type 1 cytokine storm observed in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (fig. S5, C to E) and signs of senescence in multiple tissues (Fig. 3, L to N). *Cd3e*$^{-/-}$ mice reconstituted with *Tfam*$^{fl/fl}$ *Cd4*^{Cre} BM, but not with control BM, developed a prematurely aged appearance after 16 weeks (fig. S5F) and reproduced the multimorbidity phenotype (fig. S5, G to J). Thus, T cells with mitochondrial dysfunction are able to induce systemic senescence in peripheral tissues by means of a type 1 cytokine storm.

Notably, the senescence observed in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice was accompanied by a low ratio of oxidized nicotinamide adenine dinucleotide (NAD^+) to reduced nicotinamide adenine dinucleotide (NADH) in peripheral tissues (Fig. 4A and fig. S6A). This was consistent with the reported decline in NAD^+ levels during aging (*21*). NAD^+ is a metabolic cofactor with a critical role in mitochondrial function, and restoring NAD^+ levels confers protection against age-associated diseases (*21*–*25*). Recent data suggest that supplementation with the NAD^+ precursor nicotinamide riboside (NR) reduces inflammaging in the elderly (*26*). Accordingly, we observed that NR decreased the serum levels of TNF-α in aged mice (fig. S6B). To test whether NAD^+-boosting strategies could prevent tissue damage caused by inflammaging, we treated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice with NR for 10 weeks to restore the NAD^+/NADH ratio (Fig. 4, A and B). Although NR neither prevented weight loss nor reversed the T_H1 cell phenotype (Fig. 4C), it successfully prevented skeletal muscle wasting and anemia. Furthermore, most of the cardiovascular alterations found in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice were also prevented (Fig. 4, D to G). NR treatment also increased physical activity levels of *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice and prevented tissue senescence and inflammation (Fig. 4, H to K, and fig. S6C). Finally, a principal components analysis of liver transcriptomics classified *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice treated with NR closer to controls than to untreated *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice (Fig. 4L). Ingenuity pathway analysis (IPA) linked NR treatment to pathways associated with inflammation, morbidity, and mortality (Fig. 4M and table S1). Thus, NR can prevent transcriptional changes related to aging in *Tfam*$^{fl/fl}$ *Cd4*^{Cre} mice.

With the continuous extension of life expectancy, there is an urgent need to understand the common molecular pathways by which aging results in a progressively higher susceptibility to diseases (*27*). Our results indicate that metabolic changes in the immune system promote age-related deterioration in other tissues, which leads to multimorbidity and premature death. Dysregulated T cell metabolism triggers a type 1 cytokine storm that induces senescence in several tissues. Furthermore, we have used this model of multimorbidity and premature aging to test for drugs that can delay aging signs. We found that NAD^+ precursors can prevent the tissue damage associated with sustained inflammation, which supports their potential for preventing age-associated multimorbidity. Further investigation is needed to understand whether targeting senescence or boosting NAD^+ levels could have beneficial effects beyond age-associated diseases in patients with cachexia or cytokine-release syndrome. Our results place immunometabolism at the crossroads of inflammation, senescence, and aging, thereby highlighting its potential as a therapeutic target for delaying aging and aging-associated diseases.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S6
Table S1
References (*28*–*30*)

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CHEMICAL ECOLOGY

Information arms race explains plant-herbivore chemical communication in ecological communities

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Plants emit an extraordinary diversity of chemicals that provide information about their identity and mediate their interactions with insects. However, most studies of this have focused on a few model species in controlled environments, limiting our capacity to understand plant-insect chemical communication in ecological communities. Here, by integrating information theory with ecological and evolutionary theories, we show that a stable information structure of plant volatile organic compounds (VOCs) can emerge from a conflicting information process between plants and herbivores. We corroborate this information "arms race" theory with field data recording plant-VOC associations and plant-herbivore interactions in a tropical dry forest. We reveal that plant VOC redundancy and herbivore specialization can be explained by a conflicting information transfer. Information-based communication approaches can increase our understanding of species interactions across trophic levels.

Chemical information is an ancient and ubiquitous channel to mediate species interactions (*1*) (e.g., attracting or repelling individuals) and is regarded as one of the main forces shaping plant-herbivore interaction networks (*2*, *3*). For example, insects have a large number of olfactory receptors with high sensitivity for chemical signals (*4*, *5*), which play essential roles in their fundamental activities (*6*), including foraging (*7*) and oviposition (*8*). Chemically mediated species interactions are based on information transfer, which we define simply as communication regardless of the benefit to the emitter and receiver [following previous work (*9*)]. Understanding the chemical communication between plants and herbivores has been an active field of research (*10*–*12*), and most work has focused on how a specific plant (or genus) defends against herbivores directly by using chemical repellents (*13*) or by producing stronger chemical defenses (*14*–*16*), or indirectly by emitting signals to attract herbivores' enemies (e.g., predators or parasitoids) (*17*–*22*). So far, only a few descriptive studies (*23*, *24*) have begun to investigate this chemical communication at the community level. More critically, little is known about how information transfer shapes species interactions. For example, it remains unclear how plants code chemical information to deal with a diversity of potential herbivores and how herbivores decode such olfactory signals to distinguish among plants and identify potential hosts. Even less is known about whether there is any general information structure of plant-herbivore chemical communication and how such a structure can be maintained under an ongoing chemical "arms race" between plants and herbivores.

Information theory (*25*), which provides a quantitative and scalable way to measure information transfer, has already brought key insights about the structure and emergence of human language (*26*), and it can be extended to increase our understanding about the "chemical language" in ecological communities (*12*, *27*–*29*). However, such attempts have only been used to study chemical communication of a single plant species with its interacting insects (*30*). Here, we used information theory to study plant volatile organic compounds (VOCs) as a communication channel forming plant-insect interaction networks (*31*). From an information perspective, the relationship between sender and receiver (or speaker and hearer) determines the nature of the signal transmission and the evolution of the information structure shaping the communication pattern between individuals. That is, individuals can either provide clear information that can be decoded easily or spurious information that can be difficult to decode.

Moreover, a conflicting or harmonious communication process between sender and receiver can be modeled by the combinations of maximizing or minimizing uncertainty (or mutual information) of the two parties, respectively (*26*). Specifically, for a given communication system that is formalized by a matrix describing how a vector of signals (S) is associated with a vector of objects (O), the clearness (or the spuriousness) of the communication can be described by two important information measurements (*25*). First, mutual information [$I(O,S)$] describes the amount of information that one can obtain about objects by knowing the signals. Second, conditional entropy [$H(O|S)$] describes the uncertainty of correctly identifying an object given that a specific signal has been observed [for a detailed mathematical account of these expressions, see the supplementary materials (*32*)]. Both mutual information and conditional entropy represent the efficiency of the coding (and decoding) strategies and can be related to the fitness of senders and receivers (*26*).

Tropical dry forests host a large number of interacting and coevolving plant and butterfly species and are regarded as biodiversity hotspots (*33*). Our fieldwork was conducted in a tropical dry forest of the Chamela-Cuixmala Biosphere Reserve (19°22' to 19°39' N; 104°56' to 105°10' W) in Jalisco, Mexico. During the rainy season of 2018, we searched comprehensively for lepidopteran larvae on leaves of target plants in our transect plots and reared the larvae in the laboratory with leaves collected from their host plant species to confirm their trophic interaction and to identify the herbivore species. The supplementary materials (*32*) provide a detailed account of plants and insects, sampling, and identification procedures. We constructed a qualitative (i.e., presence or absence) herbivore (animal)–plant (AP) interaction matrix comprising 28 lepidopteran herbivore species and 20 plant species. The AP matrix was rather sparse (see Fig. 1A and table S1), with a median of one plant species per herbivore (range, one to nine).

Additionally, we sampled the headspace around leaves to retrieve VOCs from each of these plant species. We were able to match 93 analytes from headspace samples to the NIST17 VOC library, of which 56 were likely biogenic (from plants or from plants and microbes), and 31 of these biogenic VOCs could be identified by one or more abundant ions in an untargeted analysis. We used single representative ions from these 31 VOCs for quantification. The supplementary materials (*32*) provide a detailed account of VOC sampling, identification, and quantification procedures. Figure 1B (and table S2) shows that, contrary to the AP matrix, the plant-VOC (PV) association matrix is dense and

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erratic, with a median of 26 VOCs per plant (range, 13 to 30).

The *PV* matrix provides information about *PV* associations, whereas the *AV* matrix (mathematically defined by the matrix multiplication $AP \times PV$) provides information about herbivore-VOC associations. These associations describe the patterns of plants emitting information and herbivores receiving it and thus represent plant coding and herbivore decoding strategies. The *AV* matrix (Fig. 1C) is dense and weighted, indicating that individual VOCs are associated with more than one herbivore. Note that to generate the *AV* matrix, we assumed that if a herbivore can feed on a plant, it must be able to cope with spatial and temporal variation in VOCs that are used to distinguish suitable hosts despite the effects of these VOCs on the herbivore (attract, deter, or neutral). Altering either the *PV* or *AP* matrix can change the *AV* matrix.

We built the conceptual framework for our study based on a conflicting optimization process between plants and herbivores (see Fig. 2). Specifically, we hypothesized that plants aim to decrease the decoding efficiency of herbivores by changing *PV* associations, whereas herbivores aim to increase this efficiency by changing *AP* interactions. Ecologically, the decoding strategy can be linked to the fitness of plants and herbivores. A high decoding efficiency can increase the attack rates and decrease the fitness of plants. By contrast, low efficiency can increase the searching time and decrease the fitness of herbivores (*12*). Formally, we define the fitness relationships as $F_P = H(A|V) \in [0,1]$ and $F_A = 1 - H(V|A) \in [0,1]$, respectively. That is, plant fitness is proportional to the conditional entropy (uncertainty) between VOCs and herbivores, whereas herbivore fitness is negatively proportional to the conditional entropy between herbivores and VOCs. The supplementary materials (*32*) provide details on the calculations.

To track the evolutionary trajectory of the communication system between plants and

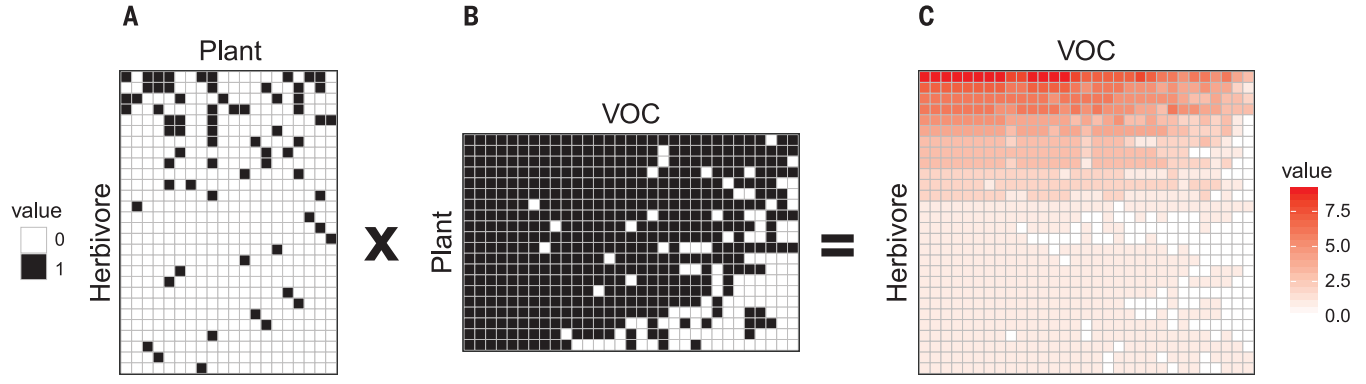

Fig. 1. Field data on *AP* interactions, *PV* associations, and *AV* associations. (**A**) Each row and column represents one of the 28 insect herbivores and 20 plants sampled, respectively. Black and white squares show that a given herbivore was or was not observed feeding on a given plant, respectively. We refer to this as the *AP* matrix and we used it to investigate the plant-herbivore interaction network. (**B**) Each row and column represents one of the 20 plants and 31 VOCs sampled, respectively. Black and white squares show that a given VOC was present or absent in a given plant, respectively. We refer to this as the *PV* matrix and we used it to investigate the coding strategy of plants. (**C**) *AV* matrix produced by the multiplication of the previous two matrices ($AP \times PV$). Each row and column represents one of the 28 herbivores and 31 VOCs associated, respectively. The darker the squares, the greater the frequency with which a given herbivore is associated with a given VOC. We refer to this as the *AV* matrix and we used it to investigate the decoding strategy of herbivores. [See the supplementary materials (*32*) and tables S1 and S2 for more information about these matrices.]

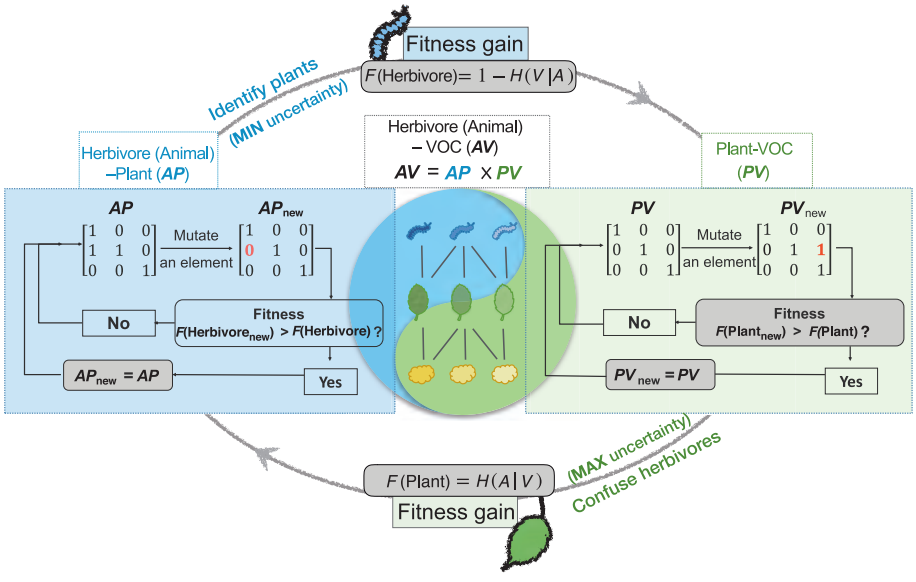

Fig. 2. Conceptual diagram of the proposed conflicting information process (information arms race) between plants and insect herbivores. The coding strategy by plants is characterized by the *PV* association matrix (green right box). The *AP* interaction matrix (blue left box) is used to infer how plants' codes are decoded by herbivores. These matrices undergo mutations by changing any of their elements (e.g., red numbers); zeros and ones correspond to absence and presence, respectively. These mutations will fixate only if the fitness relationship of the corresponding species increases. The fitness relationship of species is defined by herbivores' decoding efficiency based in the communication system, i.e., the *AV* matrix that results from the product of *AP* and *PV* matrices. Specifically, we defined plant and herbivore fitness relationships by the expressions $F_P = H(A|V)$ and $F_A = 1 - H(V|A)$, where $H(\cdot)$ corresponds to the conditional entropy. To increase this fitness relationship, plants and herbivores go through an alternating optimization process, mimicking their arms race. [For more details about this process, see the main text and the supplementary materials (*32*).]

herbivores based on our hypothesis, we simulated the optimization process above in the following way. Plants and herbivores can increase their fitness relationships (based on the *AV* matrix) by modifying the interactions in the *PV* matrix and the *AP* matrix, respectively (see Fig. 2).

These modifications come from random mutations of a given number of elements in the *PV* and *AP* matrices. We assumed an equal mutation rate for each link (*PV* link or *AP* link) in the absence of any prior knowledge. Therefore, the number of random mutations that plants and herbivores could have for each round was proportional to the number of all possible links (i.e., the number of elements in the *PV* and *AP* matrices). Mutations are only selected if they increase the corresponding fitness relationships. To mimic a continuous arms race, we only allowed changes by plants and herbivores in an alternating fashion (see Fig. 2). Both the *PV* and *AP* matrices can be initialized from any random configuration with the only restriction being matrix size. To make our simulation as simple as possible, we assumed that plants can potentially emit any VOC and herbivores can potentially eat any plant without any phylogenetic or physiological constraints.

We investigated the capacity of our hypothesized mechanism to explain both the (de)coding strategies and the information structure of chemical communication between plants and herbivores observed in the field. Specifically, we determined the ability of our model to explain the conditional entropies $H(P|V)$, $H(A|P)$, and $H(A|V)$, which characterize the *PV*, *AP*, and *AV* matrices, respectively. Then, we determined its ability to explain the observed fitness relationships F_P and F_A. Note that conditional entropy by definition is the average entropy given each specific signal [i.e., individual VOC in the case of $H(P|V)$ and $H(A|V)$]. Furthermore, we tested how accurately our model could generate the cumulative information structure given a combination of VOCs by calculating the mutual information between plants and VOCs [$I(P,V)$] and between herbivores and VOCs [$I(A,V)$] as a function of the number of VOCs.

Additionally, we compared our proposed optimization mechanism against three alternative optimization mechanisms. The fitness relationships of plants and herbivores depend on their capacity to (i) maximize uncertainty [i.e., $F_P = H(A|V)$ and $F_A = H(V|A)$], (ii) minimize uncertainty [i.e., $F_P = 1 - H(A|V)$ and $F_A = 1 - H(V|A)$], and (iii) minimize and maximize uncertainty [i.e., $F_P = 1 - H(A|V)$ and $F_A = H(V|A)$]. We also ran all our simulations over different initial conditions, ranging from highly specialized to highly generalized matrices. The rationale for using these alternative mechanisms and initializations was to illustrate that potential matches between our theoretical expectations and the observed values are not just an artifact of sample size, metrics, any given optimization process, or the high (low) connectivity observed in the *PV* (*AP*) matrix. The supplementary materials (*32*) provide all the details about these additional analyses.

Because sampling bias is an important concern in most ecological studies (*34*), we tested whether our results would be affected by the sampling effort in reporting species, by an incomplete plant VOC profile, and by sampling the interaction network in a different year or place. We found that all of our results were qualitatively equivalent when using

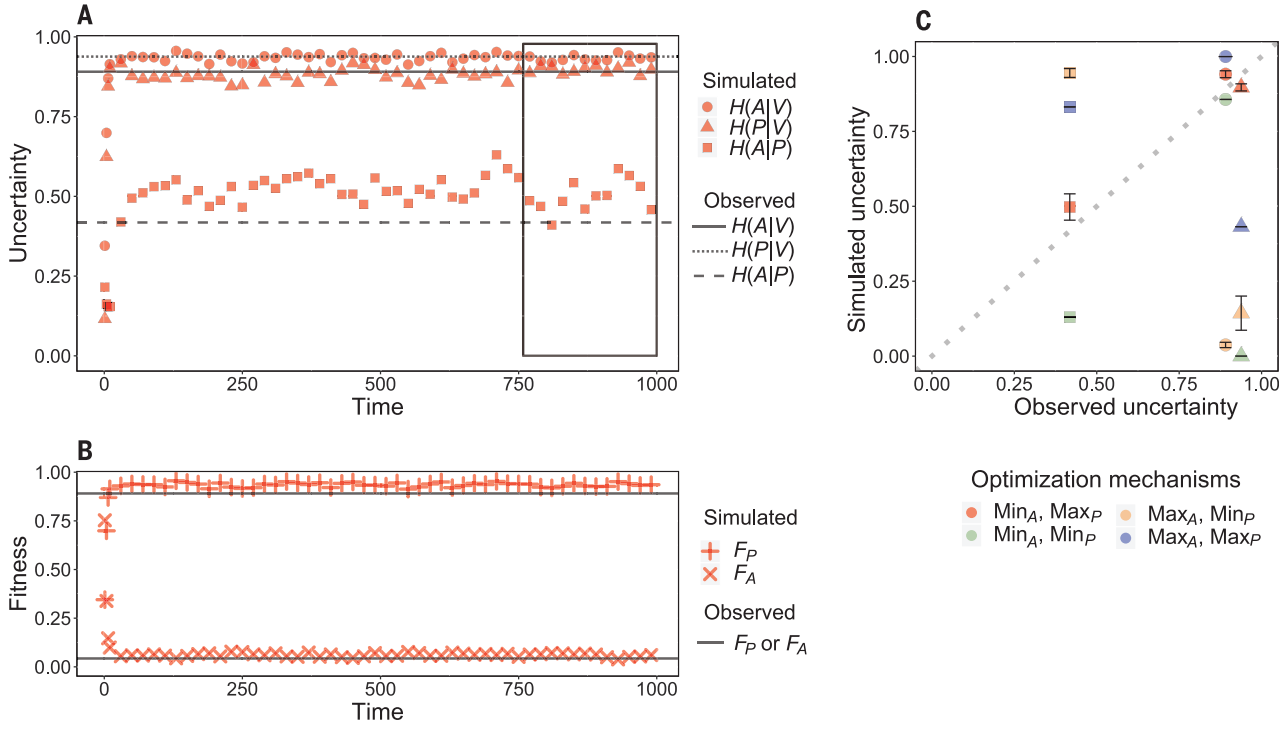

Fig. 3. Simulated results based on the information arms race theory recover the observed information structures in the field. (**A**) Simulation over 1000 time steps derived from our proposed optimization mechanism (information arms race). The simulation is randomly initialized. The circles, triangles, and squares correspond to the conditional entropies $H(A|V)$, $H(P|V)$, and $H(A|P)$, respectively. The solid, dotted, and dashed lines stand for the corresponding conditional entropies from field observations. (**B**) For the same simulation in (A), the + and × symbols correspond to the fitness relationships of plants (F_P) and herbivores (F_A), respectively. The solid lines correspond to field observations. (**C**) Summary of the observed and simulated values (mean ± SD) based on the last 250 steps [the rectangle window in (A) when reaching equilibrium for our proposed mechanism, shown in red; plants aim to maximize ($\max_A$) and herbivores aim to minimize ($\min_P$) uncertainty of information; see Fig. 2] and the corresponding ones for the three alternative mechanisms (in green, yellow, and blue; the alternative combinations of the maximization and minimization).

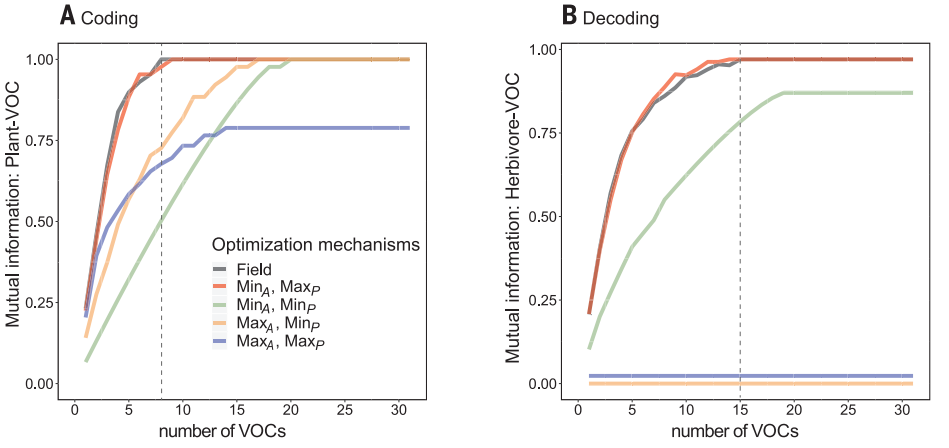

Fig. 4. Emergence of cumulative mutual information. Shown is the information structure characterized by the cumulative mutual information of (**A**) plants and VOCs [i.e., $I(P,V)$] and (**B**) herbivores and VOCs [i.e., $I(A,V)$]. The higher the value, the better the presence or absence of VOCs can identify plants. The cumulative mutual information is calculated as a function of the number of VOCs. The black curve corresponds to the field observation, and the colored curves correspond to the proposed (in red) and alternative optimization mechanisms. Dashed lines correspond to the minimum number of VOCs that gives the best mutual information according to the field data.

subsamples from the whole dataset (illustrating the scalability of our findings), when changing the VOC profile from 31 to 52 by including potential undefined biogenic VOCs, and when using information of plant-herbivore interaction networks from previous years (from 2007 to 2017) (*35*). The supplementary materials (*32*) provide all the details about the robustness of our results to changes in community size, number of VOCs, and identity and number of species interactions.

Figure 3 shows that the proposed conflicting information process is able to explain the coding and decoding patterns observed in the field. In particular, Fig. 3A shows that the conditional entropies $H(P|V)$ (triangles), $H(A|P)$ (squares), and $H(A|V)$ (circles) remain bounded across the simulated time to values close to those observed (see dashed, dotted, and solid lines, respectively). Similarly, Fig. 3B shows that the optimized fitness relationships of plants F_P (+ symbols) and herbivores F_A (× symbols) quickly converge to a stable value close to that observed (solid lines). Figure 3C shows that the alternative optimization mechanisms are not able to explain all the patterns.

Additionally, Fig. 4 shows that of our four tested models, only our proposed conflicting optimization process can recover the information structure observed in the field. Recall that we measured the cumulative information structure by the mutual information [$I(P,V)$ and $I(A,V)$], where values closer to one imply that the presence or absence of a VOC (or a combination of VOCs) gives more information or has higher probability to tell all the species apart. In fact, the field data revealed that as few as eight VOCs can tell all the plants apart and 15 VOCs can give 97% information about herbivores (i.e., have a 97% probability of correctly telling all the herbivores apart). This structure was closely recovered by our proposed optimization mechanism (nine VOCs yield all the information of plants and 14 VOCs yield 97% of information of herbivores; Fig. 4, red lines). By contrast, the three alternative optimization mechanisms yield very different information structures, where either a large number of VOCs would be needed to uniquely identify species or the identification from VOCs would be less likely. For example, in the case where both parties aim to maximize uncertainty (i.e., confuse their opponents as in a mutually competitive relationship; Fig. 4, blue lines), even by using the combination of all VOCs, it would be impossible to tell all the species apart. All these results are robust to changes in community size, number of VOCS, the identity and number of species interactions, and different initial conditions [see the supplementary materials (*32*) and figs. S1 to S6].

Although a few recent studies have begun to demonstrate chemical patterns associated with plant-insect communities (*24*, *31*, *36*), our study proposes a plausible theoretical framework that explains and recovers patterns of information transfer between plants and herbivores from empirical data. Our work is hypothesis driven and suggests that an information arms race between plants and herbivores can drive plants to produce VOCs that are commonly shared by other species, increasing the difficulty for herbivores to identify suitable plants and potentially pressuring herbivores to specialize on a few plants (as the *AP* matrix shows in Fig. 1). Indeed, previous studies have shown a substantial overlap among the VOCs emitted by different plant species (*31*, *37*, *38*). However, the functional roles of a large number of plant VOCs have been unexplained and overlooked because most mechanistic studies have focused on specific plant species and on the ecological functions of only a few VOCs (*12*). Here, our information arms race theory suggests that these seemingly redundant VOCs play an important role in confusing herbivores at the community level. Focusing on herbivore-plant interactions, herbivores are commonly regarded as specialists (*6*), which was also supported by our theory. In fact, it is estimated that <10% of all herbivores feed on plants that span more than three families (*39*).

Our findings further suggest that a conflicting information process drives the rapid accumulation of information by adding VOCs (Fig. 4), contrary to other optimization processes. This provides additional evidence for an arms race process explaining the large diversity of VOCs in nature, where herbivores have the evolutionary potential to quickly tell all plant species apart by making use of the few most informative VOCs, and plants can in turn respond to this potential by adding more VOCs to their profile. Under the same process, herbivores themselves can also be identified using a set of informative VOCs (Fig. 4B). This raises the question of how these VOC profiles result in evolutionary trade-offs regulating the attraction of herbivores and their predators and parasitoids (*17*, *18*). Overall, our study suggests that information transfer processes are key drivers of the formation and maintenance of species interactions across trophic levels.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S6
Tables S1 and S2
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Database S1
MDAR Reproducibility Checklist

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METALLOENZYMES

Structural evidence for a dynamic metallocofactor during N_2 reduction by Mo-nitrogenase

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The enzyme nitrogenase uses a suite of complex metallocofactors to reduce dinitrogen (N_2) to ammonia. Mechanistic details of this reaction remain sparse. We report a 1.83-angstrom crystal structure of the nitrogenase molybdenum-iron (MoFe) protein captured under physiological N_2 turnover conditions. This structure reveals asymmetric displacements of the cofactor belt sulfurs (S2B or S3A and S5A) with distinct dinitrogen species in the two αβ dimers of the protein. The sulfur-displaced sites are distinct in the ability of protein ligands to donate protons to the bound dinitrogen species, as well as the elongation of either the Mo–O5 (carboxyl) or Mo–O7 (hydroxyl) distance that switches the Mo-homocitrate ligation from bidentate to monodentate. These results highlight the dynamic nature of the cofactor during catalysis and provide evidence for participation of all belt-sulfur sites in this process.

Nitrogenase catalyzes a key step in the global nitrogen cycle: the ambient reduction of atmospheric dinitrogen (N_2) to the bioavailable ammonia (NH_3). The molybdenum (Mo) nitrogenase consists of two component proteins (*1*–*3*). One, designated the iron (Fe) protein, is a homodimer that contains a [Fe_4S_4] cluster at the subunit interface and an adenosine triphosphate (ATP)–binding site within each subunit. The other, designated the molybdenum iron (MoFe) protein, is an $\alpha_2\beta_2$ heterotetramer that contains two complex metalloclusters per αβ dimer: a P-cluster ([Fe_8S_7]) at each interface of the α and β subunits, and an M-cluster {or FeMoco; [(*R*-homocitrate)$MoFe_7S_9C$]} within each α subunit (*4*, *5*). Catalysis by the Mo-nitrogenase involves repeated association and dissociation between the two component proteins, which permits ATP-dependent electron transfer from the [Fe_4S_4] cluster of the Fe protein, via the P-cluster, to the M-cluster of the MoFe protein, where substrate reduction occurs (*1*–*3*). The ability of nitrogenase to shuttle multiple electrons to its cofactor renders the enzyme highly versatile in substrate reduction. Other than N_2 and protons, nitrogenase can reduce a variety of alternative substrates, such as acetylene, cyanide, and carbon monoxide (*1*, *6*, *7*).

Considerable efforts have gone into the mechanistic investigation of nitrogenase since its discovery, with a number of studies focused on the substrate and inhibitor interactions of this enzyme (*1*, *3*, *8*–*10*). However, characterization of the substrate- or intermediate-bound states of nitrogenase has proven to be challenging because of the transient nature of these bound species (*11*). To circumvent this problem, freeze-quench spectroscopic techniques, combined with strategic genetic modifications of the active site, have been applied to accumulate and characterize nitrogenase-bound intermediates, leading to the proposal of a reductive elimination mechanism (*3*) and a concurrent refinement of the Thorneley-Lowe model (*8*, *9*) for N_2 reduction by Mo-nitrogenase. Moreover, high-resolution crystal structures of Mo- and V-nitrogenases have been obtained with ligands (e.g., CO or an N or O species) bound to the enzyme (*12*, *13*), pointing to an intriguing mechanism in which a reactive Fe species is generated upon displacement of a specific belt sulfur (S2B) of the cofactor by a μ_2-bridging ligand. An elegant crystallographic pulse-chase study using a Se reporter further demonstrated a catalysis-dependent migration of belt sulfur (*14*), highlighting the dynamic nature of the entire belt region of the cofactor during turnover. Together, these studies offered invaluable insights into the reaction mechanism of nitrogenase while providing added incentive for exploration of strategies to specifically capture N_2 or related intermediate(s) for mechanistic studies.

One strategy worthy of consideration is to limit excess electron supplies that inadvertently drive the N_2 reduction forward, which either reverts the substrate- or intermediate-bound state to the resting state or renders the enzyme in an indiscernible mixed state. This is particularly relevant because nitrogenase proteins are routinely isolated in the presence of excess dithionite, an externally supplied reductant (*1*). Removal of this artificial electron source, coupled with quick isolation of the protein in the absence of oxygen, could facilitate capture of N_2 or intermediate(s) on the protein upon exhaustion

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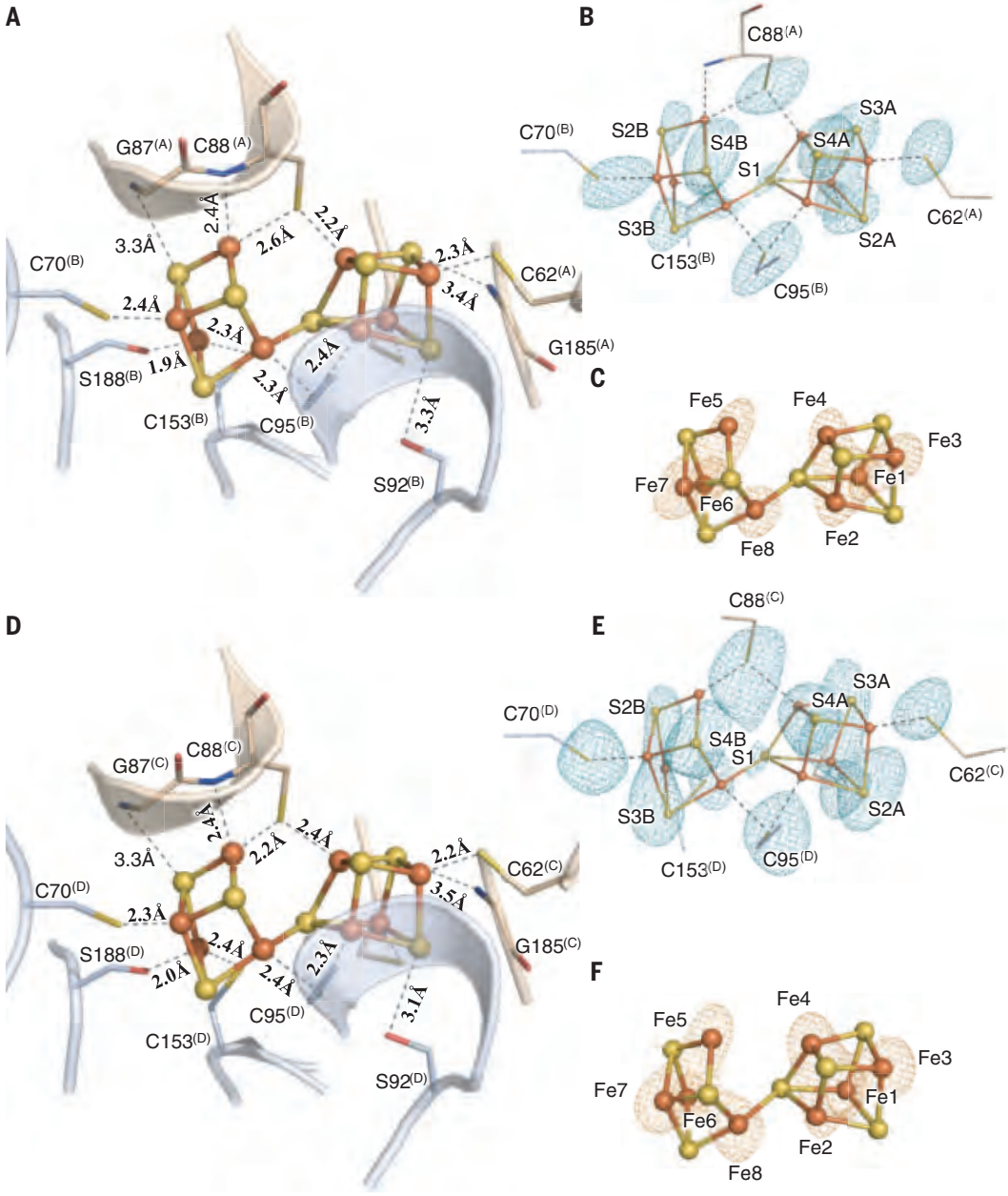

Fig. 1. The oxidized P-clusters in Av1*. Structures of P-clusters at (**A** to **C**) the interface of chains A and B [P-cluster$^{(A/B)}$] and (**D** to **F**) the interface of chains C and D [P-cluster$^{(C/D)}$] of Av1*. Chains A and C are the α subunits, and chains B and D are the β subunits of the two αβ dimers of Av1*. [(A) and (D)] The P-clusters are shown in ball-and-stick presentation, and the key residues interacting with the P-clusters are indicated as sticks. Chains A and C are shown as wheat ribbons, and chains B and D are shown as light-blue ribbons. [(B) and (C)] P-cluster$^{(A/B)}$ and [(E) and (F)] P-cluster$^{(C/D)}$ superimposed with [(B) and (E)] the anomalous density maps calculated at 7100 eV at a resolution of 2.18 Å and contoured at 4.0σ, showing the position of sulfur atoms (mint-blue mesh); and with [(C) and (F)] the anomalous density maps calculated at 7141 eV at a resolution of 2.1 Å and contoured at 15.0σ, showing the position of iron atoms (red mesh). Atoms are colored as follows: Fe, orange; S, yellow; O, red; N, blue. Single-letter abbreviations for the amino acid residues are as follows: C, Cys; G, Gly; H, His; R, Arg; S, Ser.

of available electrons. To obtain a proof of concept for this strategy, we first prepared the crude extract of an *Azotobacter vinelandii* strain under anaerobic conditions with or without addition of dithionite upon cell disruption. In both cases, the *A. vinelandii* strain was actively expressing a Mo-nitrogenase comprising a histidine-tagged MoFe protein and a nontagged Fe protein before cell disruption (*15*, *16*). Activity analysis of these samples revealed that, unlike the dithionite-treated crude extract, the dithionite-free crude extract was nearly inactive in substrate reduction, but its activity could be fully restored with the addition of dithionite and ATP (fig. S1A). This observation suggests a depletion of electrons (e.g., from ferredoxins) and other metabolites (e.g., ATP) in the crude extract when the metabolic processes that (re)generate these components are interrupted upon cell disruption. Thus, when a nitrogenase-expressing culture that is actively performing N_2 fixation is subjected to cell lysis without additional electron supplies, the nitrogenase remains functional but is potentially arrested in a "dormant" N_2- or intermediate-bound state owing to a withdrawal of electron flow to the M-cluster. Indeed, after a quick one-step purification under N_2 from the dithionite-free crude extract, the histidine-tagged MoFe protein (designated Av1*) was not only active in N_2 reduction but also fully functional upon substitution of the gas atmosphere with C_2H_2 or Ar in an in vitro activity assay where it was combined with the Fe protein (designated Av2), ATP, and dithionite (fig. S1B). Furthermore, gas chromatography–mass spectrometry analysis demonstrated that, unlike Av1 purified with dithionite from a $^{15}N_2$-grown culture (fig. S1C, black), Av1* purified without dithionite from the same $^{15}N_2$-grown culture released $^{15}N_2$ upon acid quenching (fig. S1C, red), suggesting capture of N_2 or related species on Av1*. Release of $^{15}N_2$ was not detected from the reductant-free resting-state Av1 (designated Av1′) upon incubation with $^{15}N_2$ (fig. S1C, brown), pointing to the turnover conditions as the prerequisite for capturing N_2 on Av1 while providing further support for the physiological relevance of Av1* to catalysis.

Subsequent crystallization of Av1* yielded brown crystals that diffracted to a resolution of 1.83 Å (figs. S2 to S4 and tables S1 to S3). Notably, the two P-clusters in the two αβ dimers of Av1* [designated P-cluster$^{(A/B)}$ and P-cluster$^{(C/D)}$, respectively] are no longer present in the all-ferrous resting state (P^N); instead, both clusters adopt the conformation of the previously reported two-electron oxidized state of this cluster (P^{OX}) (*17*, *18*), with the central S (designated S1) losing two Fe–S bonds and becoming μ_4-coordinated to three Fe atoms of the α-subunit cubane and one Fe atom of the β-subunit partial cubane (Fig. 1 and figs. S5 and S6). The structural rearrangement of the P-clusters in Av1*, confirmed by anomalous density data calculated at 7100 and 7141 eV, is accompanied by formation of two Fe–X bonds with an O atom of α-S188 and the backbone N atom of α-C88 (Fig. 1 and figs. S5 and S6). Electron paramagnetic resonance analysis provided further support for this assignment, showing a P^{OX}-specific [proportionality factor (g) = 11.8] signal in the spectrum of Av1* (fig. S1D) (*19*). The P^{OX} signal of Av1* is indistinguishable in intensity from that of an equimolar amount of Av1 treated with excess oxidant, suggesting that all P-clusters of Av1* exist in the P^{OX} state (fig. S1D). The presence of the P-clusters of Av1* in the P^{OX} state not only provides the long-sought-after answer to the physiological relevance of this state, but it also points to a limited electron flow from the P-cluster to the M-cluster owing to the absence of dithionite, leaving the former in an electron-depleted state while permitting the latter to capture N_2 and/or intermediate(s) under limited turnover conditions.

Consistent with this suggestion, in the α subunit (designated chain A) of one αβ dimer of Av1*, the absence of a belt S (S2B) from the M-cluster [designated M-cluster$^{(A)}$] can be clearly and reproducibly visualized in the anomalous difference Fourier map calculated with diffraction data at 7100 eV (Fig. 2, A and B, and fig. S7, A and B); yet, based on analysis of the $2F_o$-F_c and F_o-F_c maps (fig. S8A), there is electron density at this location, which can be modeled as a dinitrogen species that binds in a pseudo $\mu_{1,2}$-bridging mode between Fe2 and Fe6 (Fig. 2C; see figs. S9 and S10 for ligand modeling). The proximal (N_{prox}) and distal (N_{dist}) nitrogen atoms of this species are located at a distance of 1.8 and 2.3 Å, respectively, from Fe2 and Fe6, rendering this dinitrogen species roughly parallel to the M-cluster along the Fe1–C–Mo axis (Fig. 2C and table S3). Additionally, N_{prox} interacts with the Nε group of α-H195$^{(A)}$, a residue implicated in N_2 reduction (*20*), via hydrogen bonding at 2.9 Å (Fig. 2C). Sulfur anomalous density [designated S$^{(A/B)}$] is observed at the same sulfur-binding site (SBS) at the interface of chains A and B as that identified earlier in the S2B-displaced, CO-bound structure of the M-cluster (*12*). Located ~22 Å away from the S2B site in chain A of Av1* (fig. S11, A and B), the relevance of this sulfur species to the displaced S2B sulfur is yet to be established. Displacement of S2B with the dinitrogen species, like that of S2B with CO, does not have much impact on the structure of the M-cluster, as both ligand-bound clusters show an overall conservation of the Fe–S, Fe–Fe, and Mo–S distances as those in the resting-state cofactor (fig. S12 and table S3) (*5*). However, contrary to its CO-bound and resting-state counterparts

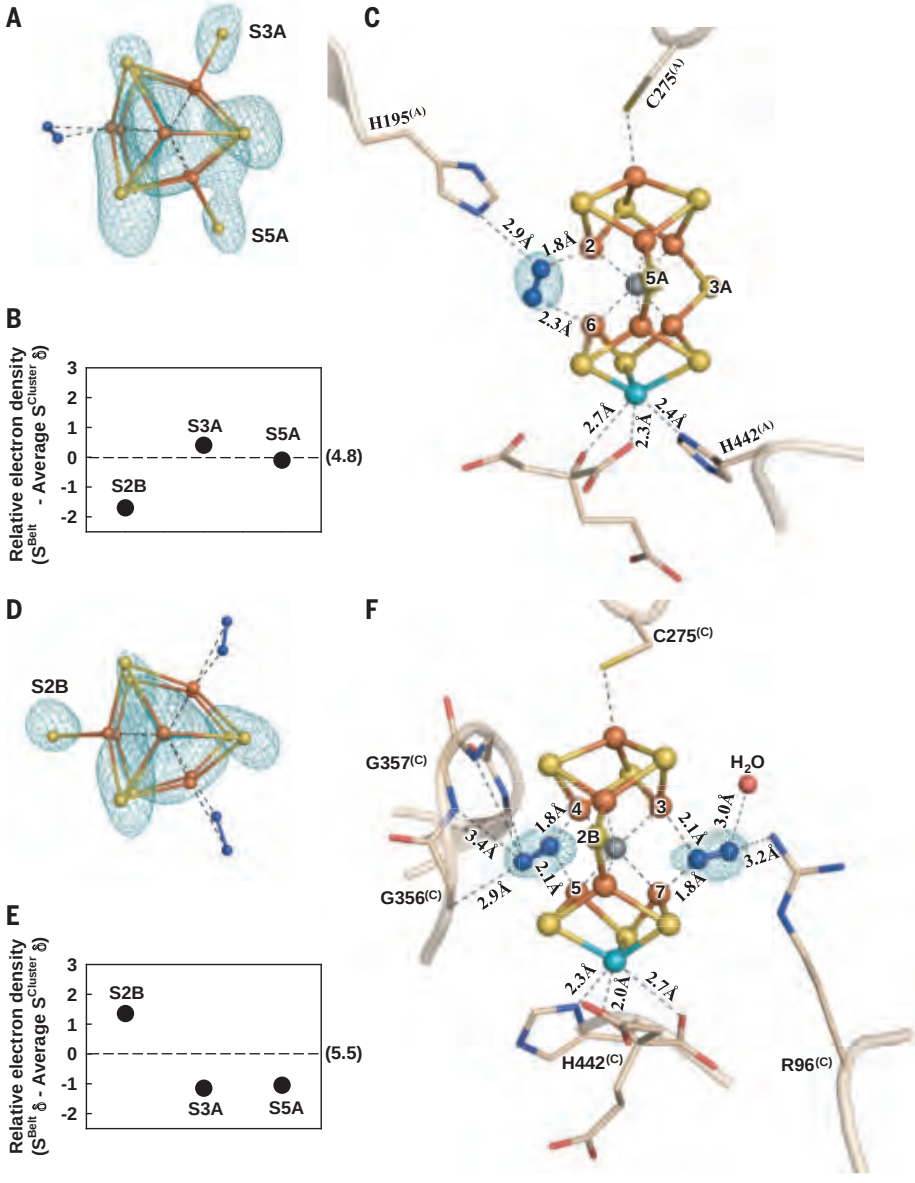

Fig. 2. The nitrogen ligand–bound M-clusters in Av1*. Structures of M-clusters in (**A** to **C**) chain A [M-cluster$^{(A)}$] and (**D** to **F**) chain C [M-cluster$^{(C)}$] refined at a resolution of 1.83 Å. View along the Fe1-C-Mo direction of (A) M-cluster$^{(A)}$ and (D) M-cluster$^{(C)}$ superimposed with the anomalous density maps (mint-blue mesh) calculated at 7100 eV at a resolution of 2.18 Å and contoured at 4.0σ (A) and 5.3σ (D), respectively, showing the displacement of sulfur at the S2B site in M-cluster$^{(A)}$ (A) and at the S3A and S5A sites in M-cluster$^{(C)}$ (D). Electron densities of the belt sulfurs (S^{Belt}) relative to the average density of cluster sulfurs ($S^{Cluster}$) in (B) chain A and (E) chain C, respectively, expressed in sigma values. The average sigma values of the cluster sulfurs in chains A and C are 4.8 and 5.5, respectively [(B) and (E)]. Side view of (C) M-cluster$^{(A)}$ and (F) M-cluster$^{(C)}$ with key residues interacting with the clusters and the bound nitrogen ligands indicated as sticks. M-cluster$^{(A)}$ and M-cluster$^{(C)}$ are superimposed with the F_o-F_c omit map of the nitrogen ligands contoured at 10σ (mint-blue mesh). The peptides and atoms are colored as in Fig. 1.

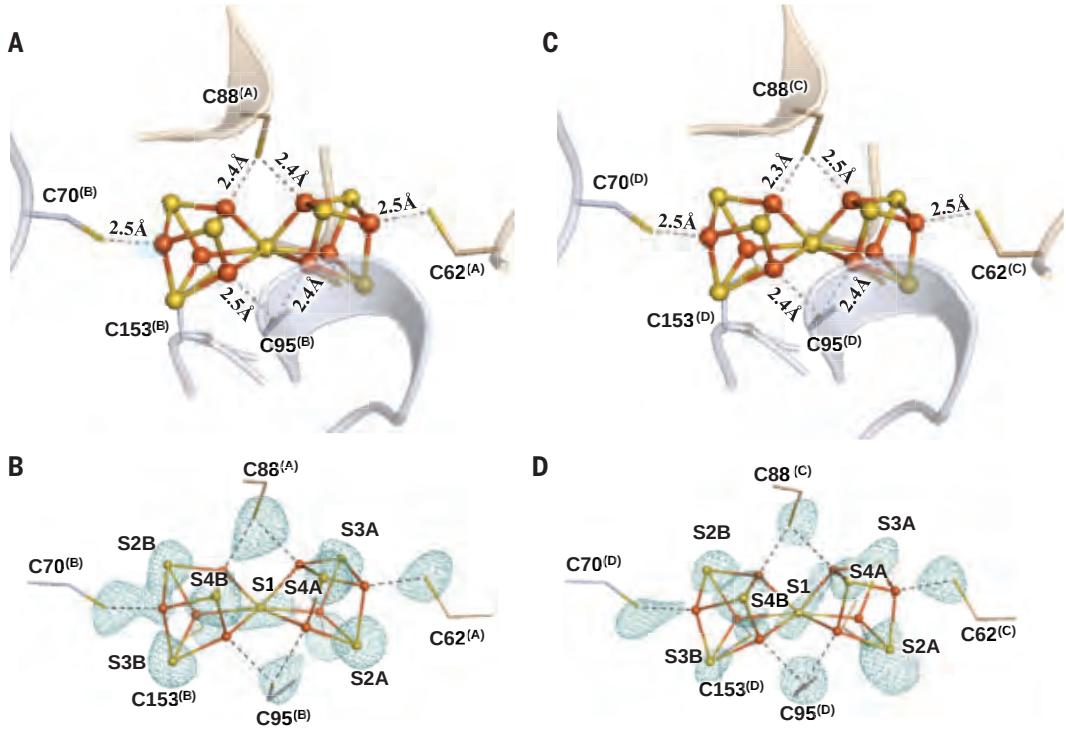

Fig. 3. The reduced P-clusters in Av1*(TOD). Structures of P-clusters at (**A** and **B**) the interface of chains A and B [P-cluster$^{(A/B)}$] and (**C** and **D**) the interface of chains C and D [P-cluster$^{(C/D)}$] of Av1*(TOD). Chains A and C are the α subunits, and chains B and D are the β subunits of the two αβ dimers of Av1*(TOD). [(B) and (D)] P-clusters superimposed with the anomalous density maps calculated at 7100 eV at a resolution of 2.17 Å and contoured at 4.5σ, showing the position of sulfur atoms (mint-blue mesh). The peptides and atoms are colored as in Fig. 1.

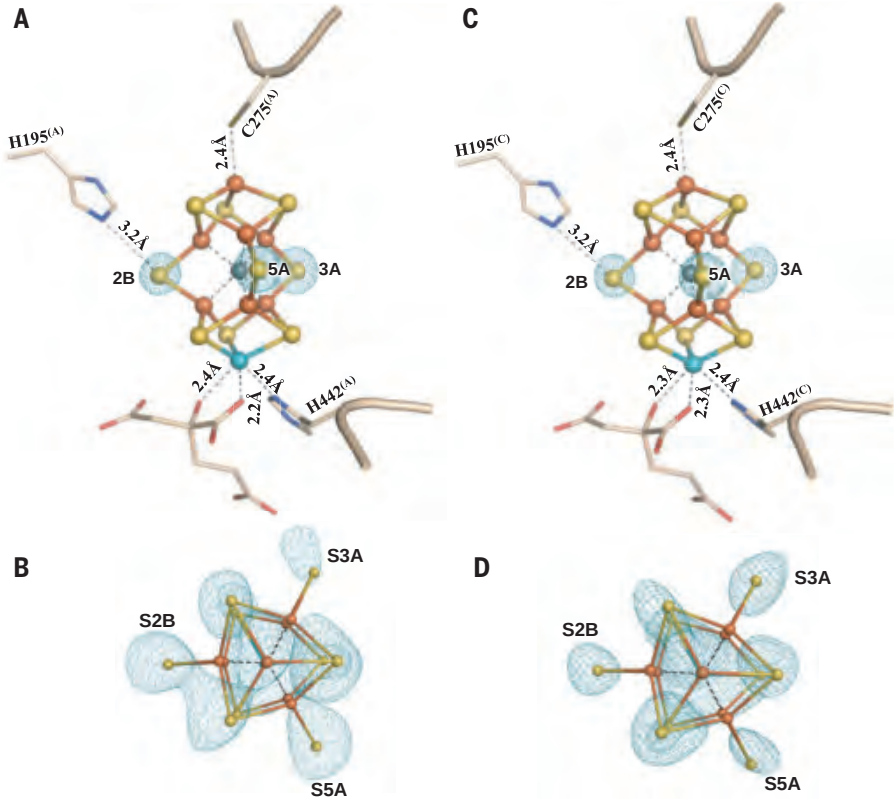

Fig. 4. The M-clusters in Av1*(TOD). Structures of M-clusters in (**A** and **B**) chain A [M-cluster$^{(A)}$] and (**C** and **D**) chain C [M-cluster$^{(C)}$] refined at a resolution of 1.73 Å. Side view of (A) M-cluster$^{(A)}$ and (C) M-cluster$^{(C)}$ with key residues interacting with the clusters indicated as sticks. M-cluster$^{(A)}$ and M-cluster$^{(C)}$ are superimposed with the F_o-F_c omit maps of the belt sulfurs contoured at 13σ (mint-blue mesh). View along the Fe1-C-Mo direction of (B) M-cluster$^{(A)}$ and (D) M-cluster$^{(C)}$ superimposed with the anomalous density maps calculated at 7100 eV at a resolution of 2.17 Å and contoured at 4.0σ, showing the presence of the anomalous sulfur density (mint-blue mesh) at all belt-sulfur positions (S2B, S3A, and S5A) in (B) M-cluster$^{(A)}$ and (D) M-cluster$^{(C)}$. The peptides and atoms are colored as in Fig. 1.

(*12*), there is a considerable change in the ligation of Mo by homocitrate in chain A of Av1*. Whereas the Mo–O5 (carboxyl) distance remains largely unchanged, there is a notable elongation of the Mo–O7 (hydroxyl) distance, thereby switching the ligation of Mo by homocitrate from bidentate in the resting-state and CO-bound structures (Mo–O5, 2.2 Å; Mo–O7, 2.2 Å) to monodentate in chain A of Av1* (Mo–O5, 2.3 Å; Mo–O7, 2.7 Å) (Fig. 2C).

Unexpectedly, in the M-cluster [designated M-cluster$^{(C)}$] of the α subunit (designated chain C) of the second αβ dimer, sulfur is clearly and reproducibly visualized at the S2B position but not at the S3A and S5A positions in the anomalous difference Fourier map calculated from diffraction data at 7100 eV (Fig. 2, D and E, and fig. S7, C and D). Like the

S2B position in chain A, the electron densities observed at the S3A and S5A positions on the basis of analysis of the $2F_o$-F_c and F_o-F_c maps of chain C (fig. S8B) can each be modeled with a dinitrogen species with N_{prox} and N_{dist} relative to the cluster Fe atoms (Fig. 2F, and figs. S9 and S10). At the S3A site, N_{prox} is located 1.8 and 2.1 Å, respectively, from Fe4 and Fe5; whereas at the S5A site, N_{prox} is located 2.1 and 1.8 Å, respectively, from Fe3 and Fe7 (Fig. 2F and table S3). As such, the dinitrogen species at S3A or S5A appears to form an asymmetric $\mu_{1,1}$ bridge between two Fe centers, with a stronger (1.8 Å) and a weaker (2.1 Å) interaction of N_{prox} with a primary (Fe4 or Fe7) and a secondary (Fe5 or Fe3) Fe center. In both cases, the distal nitrogen atoms are directed away from the M-cluster, rendering the two nitrogen atoms in a somewhat linear alignment with the primary Fe center. Besides interacting with the cluster, the dinitrogen species at the S3A site has potential hydrogen bonding interactions with the amide groups of the protein backbone [α-G356$^{(C)}$ and α-G357$^{(C)}$; N_{dist}–N_{amide} = 2.9–3.4 Å); whereas the dinitrogen species at the S5A site has potential hydrogen bonding interactions with the side chain of α-R96$^{(C)}$ (N_{dist}–N_{R96} = 3.2 Å), a residue implicated in N_2 reduction (*21*), and a water molecule (N_{dist}–O_{H2O} = 3.0 Å) (Fig. 2F). As observed at the interface of chains A and B, sulfur anomalous density [designated S$^{(C/D)}$] appears at the same SBS at the interface of chains C and D, which is ~19 and 25 Å, respectively, away from the S5A and S3A sites (fig. S11, C and D). Additionally, the M-cluster in chain C adopts a conformation that closely resembles the resting-state structure (fig. S13) (*5*). Finally, there is also a clear switch of the ligation of Mo by homocitrate from bidentate to monodentate in chain C. However, the two Mo–O distances in chain C undergo changes opposite to those in chain A, with the Mo–O5 (carboxyl) distance lengthened to 2.7 Å and the Mo–O7 (hydroxyl) distance shortened to 2.0 Å (Fig. 2F).

To further validate the sulfur-displaced conformation captured in Av1* under a limited electron flux, Av1* was incubated with Av2, ATP, and excess dithionite and reisolated from the incubation mixture. Crystallization of this reisolated Av1 protein [designated Av1*(TOD); TOD, turnover with dithionite] yielded brown crystals that diffracted to a resolution of 1.73 Å (figs. S14 and S15 and tables S1, S3, and S4). Both P-clusters of Av1*(TOD) adopt the conformation of the reduced P^N state (Fig. 3 and figs. S16 and S17); whereas both M-clusters of Av1*(TOD) adopt the resting-state conformation, with all three belt sulfurs in place and homocitrate assuming bidentate ligation to Mo via O5 and O7 (Fig. 4 and figs. S18 and S19). These observations are consistent with the bound dinitrogen species being turned over and subsequently released from the M-clusters, as well as the inability of the M-clusters to (re)capture N_2 or N_2-derived intermediates in the presence of excess reductant, which inadvertently reverts both P- and M-clusters in Av1*(TOD) to the well-established resting-state conformations. The ability of Av1* to resume turnover further illustrates the physiological relevance of this conformation to catalysis. Moreover, the "return" of belt sulfurs to both M-clusters in Av1*(TOD) provides strong validation for the asymmetric displacement of the belt sulfurs in the two M-clusters of Av1*.

Our observation that the S2B, S3A, and S5A sites can each be displaced by a dinitrogen species is consistent with the previously observed "migration" of a Se reporter in the entire belt region upon turnover (*14*) and points to a possible involvement of all three sites in N_2 reduction. At the S3A site, the backbone amides of α-G356$^{(C)}$ and α-G357$^{(C)}$ that interact with the dinitrogen species are unlikely to serve as proton donors for N_2 reduction. At the S2B site, however, there is a hydrogen bond between α-H195$^{(A)}$ and the dinitrogen species that could provide protons for N_2 reduction. Additionally, an elongation of the Mo–O7 (hydroxyl) distance to 2.7 Å would be consistent with a protonation event that breaks this bond in exchange for N_2 reduction. At the S5A site, the dinitrogen species seems primed to accept protons from hydrogen bonds with α-R96$^{(C)}$ and a nearby water molecule. Moreover, an elongation of the Mo–O5 (carboxyl) distance to 2.7 Å allows O5 to participate in a hydrogen bond with a nearby water molecule that is positioned at 2.5 Å from O5 and ~4 Å from the S5A site. With ample proton sources available, the dinitrogen species at S2B and S5A are likely more protonated and/or reduced than N_2. Notably, the binding conformation of dinitrogen species at S2B is similar to the cis-$\mu_{1,2}$-binding mode of a diazene adduct to a synthetic compound (*22*, *23*). Moreover, modeling of allowable N–N bond distances suggests the possibility of having diazene-level species bound at both the S2B and S5A sites while disfavoring the presence of a hydrazine-level species at the S5A site (fig. S9). Although many mechanisms can be proposed to explain our observations (figs. S20 to S24; also see discussion in supplementary materials), experimental support is yet to be acquired for any of these proposals. The possibility that all belt-sulfur sites are involved in catalysis, stemming from our observation of asymmetric belt-sulfur displacements in the two αβ dimers of Av1, should provoke a (re)calibration in the mechanistic thinking of nitrogenase, with the ultimate goal of elucidating the intricate mechanism of enzymatic N_2 reduction.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S24
Tables S1 to S4
References (*25*–*45*)
MDAR Reproducibility Checklist

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PHASE SEPARATION

Partitioning of cancer therapeutics in nuclear condensates

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The nucleus contains diverse phase-separated condensates that compartmentalize and concentrate biomolecules with distinct physicochemical properties. Here, we investigated whether condensates concentrate small-molecule cancer therapeutics such that their pharmacodynamic properties are altered. We found that antineoplastic drugs become concentrated in specific protein condensates in vitro and that this occurs through physicochemical properties independent of the drug target. This behavior was also observed in tumor cells, where drug partitioning influenced drug activity. Altering the properties of the condensate was found to affect the concentration and activity of drugs. These results suggest that selective partitioning and concentration of small molecules within condensates contributes to drug pharmacodynamics and that further understanding of this phenomenon may facilitate advances in disease therapy.

The five to 10 billion protein molecules of cells are compartmentalized into both membrane-bound and non–membrane-bound organelles (*1*–*3*). Many non–membrane-bound organelles are phase-separated biomolecular condensates with distinct physicochemical properties that can absorb and concentrate specific proteins and nucleic acids (*4*–*17*). We reasoned that selective condensate partitioning might also occur with small-molecule drugs with targets that occur within condensates (Fig. 1A), and that the therapeutic index and efficacy of such compounds might therefore relate to their ability to partition into condensates that harbor their target. To test this idea, we focused our study on a collection of nuclear condensates previously reported in cell lines, demonstrated that they all occur in normal human cells and in tumor cells, and then developed in vitro condensate droplet assays with key components of each of the nuclear condensates to enable testing of small molecules.

Nuclear condensates have been described in diverse cultured cell lines and contain one or more proteins that can serve both as markers of the condensate and as a scaffold for condensate formation in droplet assays in vitro (*10*–*12*, *18*–*31*). Specifically, transcriptional condensates are marked by the condensate-forming proteins MED1 and BRD4 (*10*, *12*, *19*), splicing speckles by SRSF2 (*11*, *20*), heterochromatin by HP1α (*21*, *22*), and nucleoli by FIB1 and NPM1 (*23*–*25*) (fig. S1A). To determine whether such condensates can also be observed in the cells of healthy and malignant human tissue, we obtained biopsies of breast ductal epithelium, invasive ductal carcinoma, normal colon, and colon cancer (fig. S1, B and C). Immunofluorescence revealed nuclear bodies containing these marker proteins in both normal and transformed tissue (Fig. 1, B and C). There was a broad distribution of nuclear body sizes and numbers, as expected for dynamic biomolecular condensates, and no significant differences were observed between benign and malignant tissue (fig. S2, A to C). However, tumor cells acquire large superenhancers (SEs) at driver oncogenes (*32*) and these can form tumor-specific transcriptional condensates.

We developed an assay to model these nuclear condensates and study the behavior of small molecules within these droplets (Fig. 1D). We produced and purified recombinant, fluorescently labeled versions of MED1, BRD4, SRSF2, HP1α, FIB1, and NPM1 (fig. S3, A and B) and confirmed the ability of these proteins to form droplets in an in vitro assay (fig. S4, A and B). To investigate the partitioning behavior of small molecules, we added the dyes fluorescein (332 Da) and Hoechst (452 Da), as well as fluorescently labeled dextrans (4.4 kDa), to solutions containing each of the six protein condensates. The dyes and dextrans appeared to diffuse through all the condensates without substantial partitioning (Fig. 1E and figs. S5 and S6, A to D). Small-molecule drugs are generally smaller than 1 kDa, so these results suggested that small molecules can freely diffuse through these nuclear condensates unless there are factors other than size that influence partitioning.

We next sought to determine whether diverse clinically important drugs with targets that reside in nuclear condensates also exhibit free diffusion across these condensates or if they display a different behavior. Cisplatin and mitoxantrone, members of a class of antineoplastic compounds that modify DNA through platination or intercalation, can either be modified to have fluorescent properties (cisplatin) (*33*) or are already inherently fluorescent (mitoxantrone). When added to droplet formation buffer with purified MED1, BRD4, SRSF2, HP1α, FIB1, or NPM1, cisplatin was found to be selectively concentrated in MED1 droplets (Fig. 2A and fig. S7A), with a partition coefficient of up to 600 (fig. S8, A to C). Fluorescent modification of cisplatin did not appear to contribute to this behavior in vitro, because the modified drug could be chased out of the condensate with unmodified cisplatin, and an isomer of cisplatin did not exhibit the same behavior (fig. S7, B to D). Mitoxantrone was also concentrated in MED1 condensates, as well as in FIB1 and NPM1 condensates (Fig. 2B and fig. S7A). Consistent with these results, mitoxantrone is known to concentrate in the nucleolus, where FIB1 and NPM1 reside (*34*, *35*). These results show that, in contrast to the dyes tested above, small-molecule drugs may concentrate in certain condensates even in the absence of the drug target.

We selected for further study antineoplastic drugs that target transcriptional regulators

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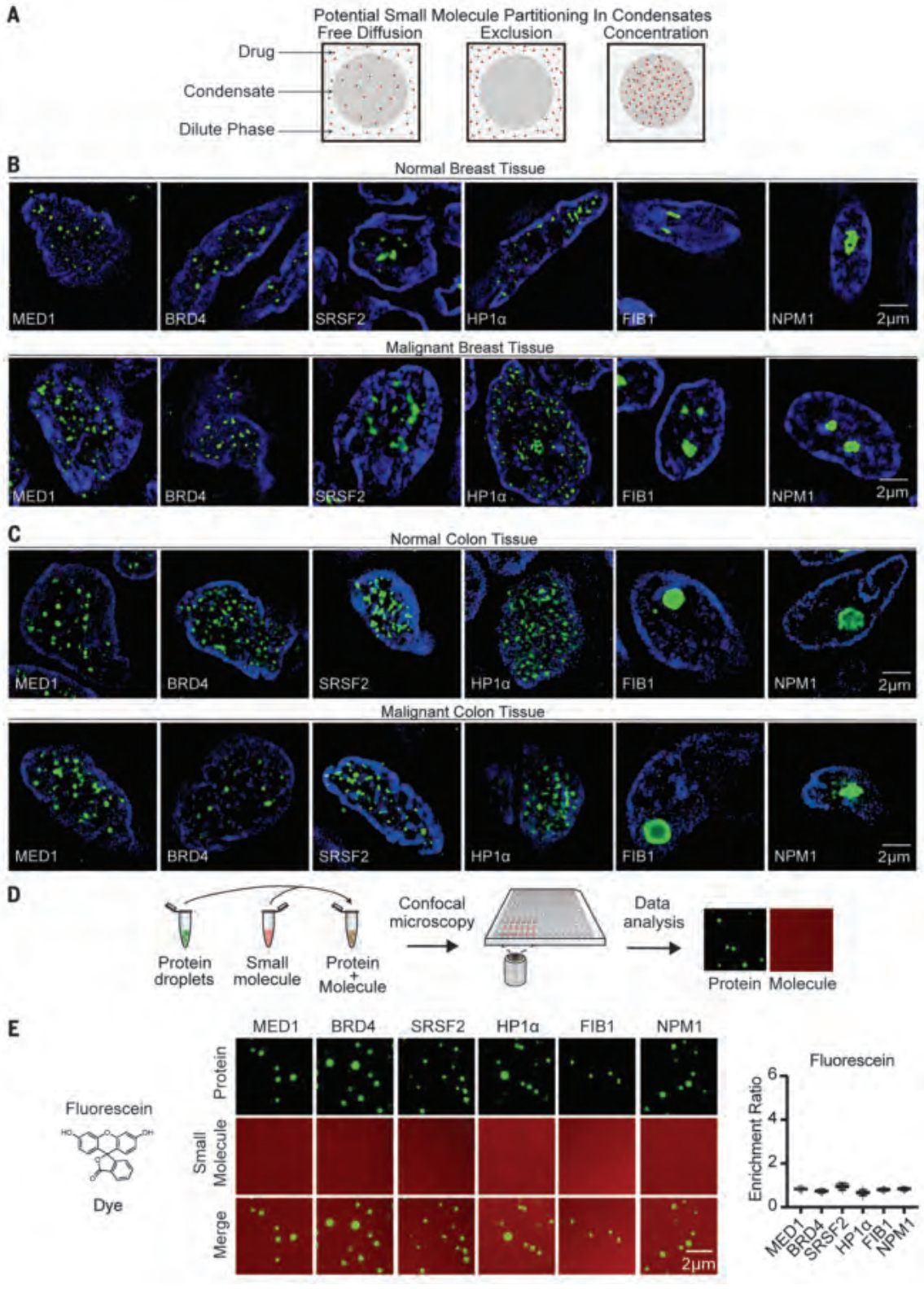

Fig. 1. Nuclear condensates in human tissue and in vitro. (**A**) Model illustrating the potential behaviors of small molecules in nuclear condensates. (**B** and **C**) Immunofluorescence of scaffold proteins of various nuclear condensates in tissue biopsies from benign and malignant human breast tissue (B) and from benign and malignant colon tissue (C) in nuclei stained with Hoechst and imaged at 100× on a fluorescent confocal microscope (see also figs. S1 and S2). (**D**) Schematic of in vitro droplet formation assay to measure small-molecule partitioning into nuclear condensates. (**E**) In vitro droplet assay showing the behavior of fluorescein dye in the presence of six protein condensates formed in 125 mM NaCl and 10% PEG with 10 μM protein and 5 μM fluorescein imaged at 150× on a confocal fluorescent microscope (see also figs. S3 to S6). Quantification of enrichment of the drug is shown on the right. Error bars represent SEM.

expected to be contained within transcriptional condensates in cells. These targets include: (i) the estrogen receptor (ER), a transcription factor and nuclear hormone receptor; (ii) CDK7, a cyclin-dependent kinase that functions in transcription initiation and cell cycle control; and (iii) BRD4, a bromodomain protein and coactivator involved in oncogene regulation (fig. S9, A and B). To monitor drug behavior with a confocal fluorescent microscope, we used a fluorescent tamoxifen analog (FLTX1) that targets ER and modified fluorescent THZ1 and JQ1, which target CDK7 and BRD4, respectively (*36*, *37*). FLTX1 and THZ1 concentrated preferentially in MED1 droplets (Fig. 2, C and D, and fig. S7A), and this behavior was not attributable to the fluorescent moiety (fig. S7, B and D). JQ1 concentration presented a different pattern, being concentrated in MED1, BRD4, and NPM1 droplets (Fig. 2E and fig. S7, A and B). Reinforcing these results, we found that the small molecules that concentrated in MED1 condensates were also concentrated in condensates formed from purified whole Mediator complexes (fig. S10A) and in MED1 condensates formed in an alternative crowding agent (fig. S11A). The targets of these three compounds (ERα, CDK7, and the bromodomains of BRD4) are not present in these in vitro condensates but are present in the SEs that form condensates with transcription factors and Mediator in vivo (*10*, *12*, *38*) (fig. S9, A and B), suggesting that the ability of some small molecules to concentrate preferentially in the same condensate as their protein target may contribute to the pharmacological properties of these drugs.

To gain additional insight into the nature of interactions governing small-molecule enrichment in condensates, we focused on the MED1-IDR condensate. Fluorescence recovery after photobleaching (FRAP) experiments showed that cisplatin molecules were highly mobile in this condensate (fig. S12, A and B), suggesting that the condensate produces a physicochemical environment that facilitates drug concentration in a state of high dynamic mobility. To gain insights into the chemical features of small molecules that may contribute to selective association with MED1 in condensates, we used a fluorescent boron-dipyrromethene (BODIPY) library of 81 compounds with various combinations of chemical side groups (fig. S13A). Molecules that contained aromatic rings were found to preferentially concentrate in MED1 condensates (figs. S13, A to D, and S14A). These data suggest that pi–pi or pi–cation interactions are among the physicochemical properties that favor small-molecule partitioning into MED1 condensates. Aromatic amino acids participate in pi–system interactions and are overrepresented in the MED1 IDR relative to the other condensate-forming proteins studied (fig. S3B). We generated a MED1 aromatic mutant protein (all 30 aromatic amino acids mutated to alanine) that retained the ability to form droplets in vitro, indicating that the aromatic amino acids are not required for droplet formation (fig. S14, B and C), but small-molecule probes containing aromatic rings and the polar molecule cisplatin no longer partitioned into condensates

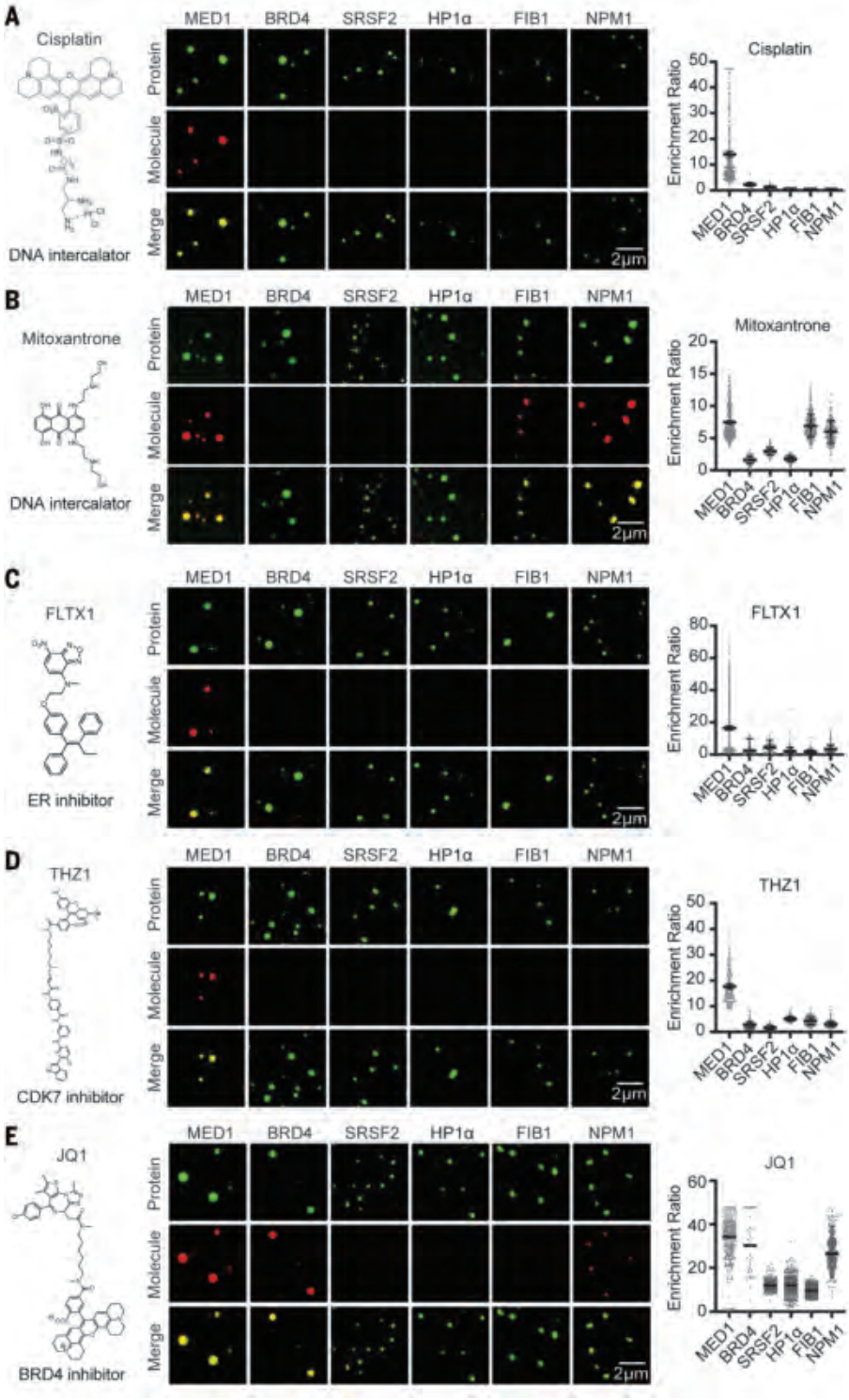

Fig. 2. Partitioning behavior of small-molecule drugs in nuclear condensates in a droplet assay. Six nuclear condensates formed in 125 mM NaCl and 10% PEG with 10 μM protein treated with (**A**) 5μM cisplatin-TMR, (**B**) 50 μM mitoxantrone, (**C**) 100 μM FLTX1, (**D**) 5 μM THZ1-TMR, or (**E**) 1 μM JQ1-ROX imaged at 150× on a confocal fluorescent microscope (see also figs. S7 to S11). Quantification of enrichment of the drug within droplets is shown on the right of each panel. Error bars represent SEM (see also figs. S12 to S14).

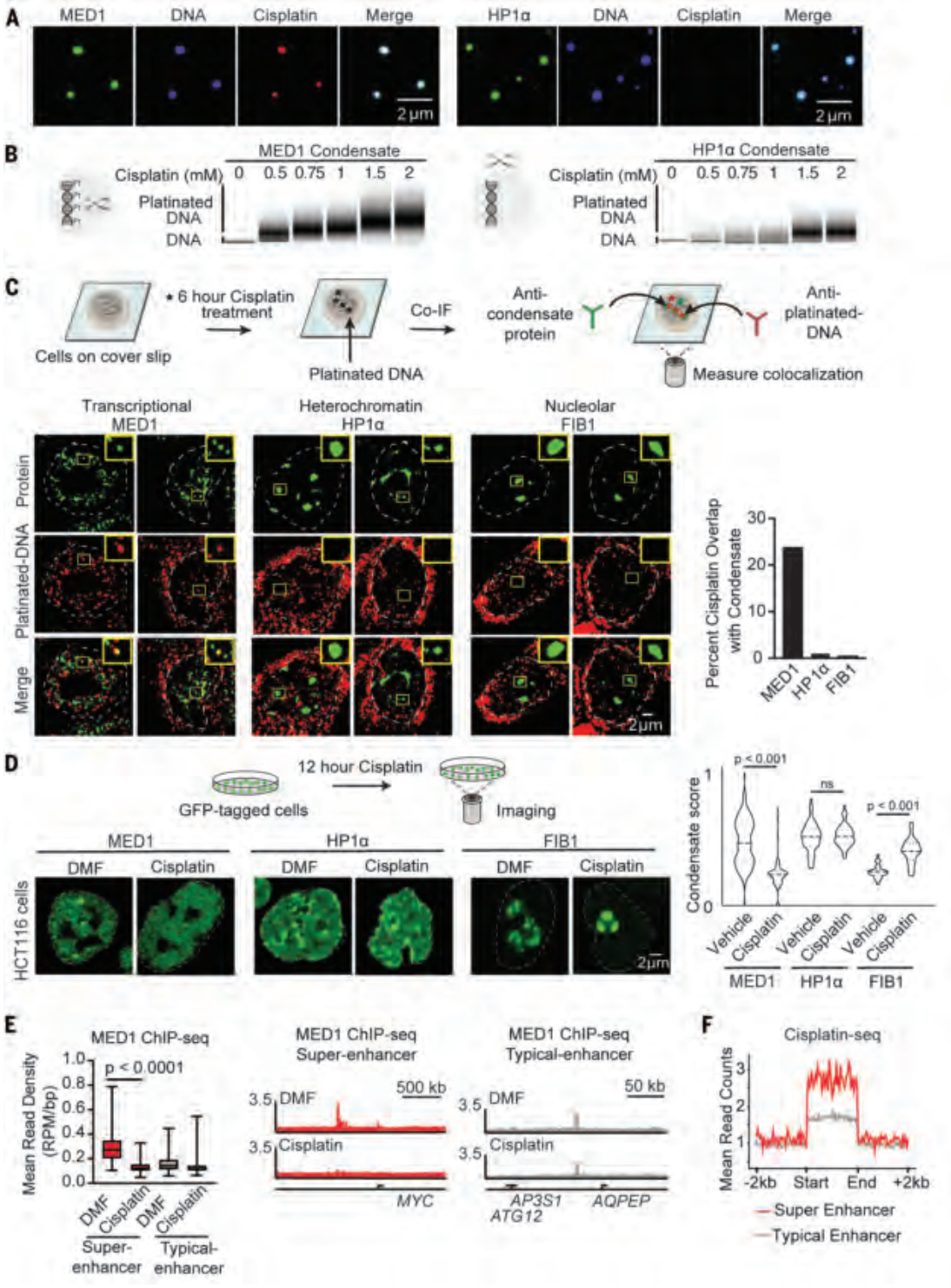

Fig. 3. Small-molecule concentration within condensates influences drug activity. (**A**) In vitro droplet assay of MED1 and HP1α condensates formed in 125 mM NaCl and 10% PEG, 5 nM of 450-bp DNA, 10 μM MED1, and 5 μM cisplatin-TR imaged at 150× on a confocal fluorescent microscope (see also fig. S15). (**B**) Bioanalyzer tracings of DNA contained within either MED1 or HP1α droplets exposed to the indicated concentration of cisplatin. (**C**) (Top) Schematic of an assay to determine the location of platinated DNA relative to various nuclear condensates. (Bottom) Coimmunofluorescence of platinated DNA and the indicated protein in HCT116 cells treated with 50 μM cisplatin for 6 hours imaged at 100× on a confocal fluorescent microscope. Quantification of overlap is shown on the right. (**D**) (Top) Schematic of a live-cell condensate dissolution assay. (Bottom) HCT116 cells bearing endogenously mEGFP-tagged MED1, HP1α, or FIB1 treated with 50 μM cisplatin for 12 hours. Quantification of MED1, HP1α, or FIB1 condensate score is shown on the right. (**E**) MED1 ChIP-seq in HCT116 cells treated with vehicle or 50 μM cisplatin for 6 hours. (Left) Mean read density of MED1 at SEs and typical enhancers. Error bars represent minimum and maximum. (Right) Gene tracks of MED1 ChIP-Seq at the *MYC* SE and AQPEP typical enhancer. (**F**) Metaplot of cisplatin-DNA-Seq in cisplatin-treated HeLa cells comparing SEs and typical enhancers (*40*) (see also figs. S16 to S21).

formed by the MED1 aromatic mutant protein (fig. S14, D to F). These results suggest that the aromatic residues of MED1 condensates contribute to the physicochemical properties that selectively concentrate these small molecules.

We anticipated that the ability of small molecules to concentrate in specific condensates would influence target engagement and thus drug pharmacodynamics. To investigate this, we took advantage of the ability of condensates to incorporate DNA (Fig. 3A and fig. S15A) and measured the relative efficiency of DNA platination by cisplatin in MED1 condensates, where cisplatin is concentrated, versus HP1α condensates, where cisplatin freely diffuses (Fig. 2A). DNA platination, visualized by size shift on a bioanalyzer, was more prevalent in MED1 condensates than in HP1α condensates (Fig. 3B), consistent with the expectation that elevated concentrations of cisplatin in the MED1 condensates yield enhanced target

engagement. If cisplatin becomes concentrated in Mediator condensates in cells, then we would expect that DNA colocalized within Mediator condensates would be preferentially platinated. To test this idea, we performed coimmunofluorescence in cisplatin-treated HCT116 colon cancer cells using an antibody that specifically recognizes platinated DNA (fig. S16A) (*39*), together with antibodies specific for MED1, HP1α, or FIB1. Consistent with cisplatin's preference for MED1 condensates in vitro, we found that platinated DNA frequently colocalized with MED1 condensates but not with HP1α or FIB1 condensates (Fig. 3C). To determine whether the ability of cisplatin to engage DNA is dependent on the presence of a MED1 condensate, we treated cells with JQ1, which caused a loss of MED1 condensates (fig. S16B), and observed a concomitant reduction in platinated DNA at the *MYC* oncogene (fig. S16, C and D). These results are consistent with the idea that the concentration of small molecules in specific condensates can influence the efficiency of target engagement.

In cells, the preferential modification of DNA in MED1-containing condensates might be expected to selectively disrupt these condensates with prolonged treatment. To test this, HCT116 colon cancer cells were engineered

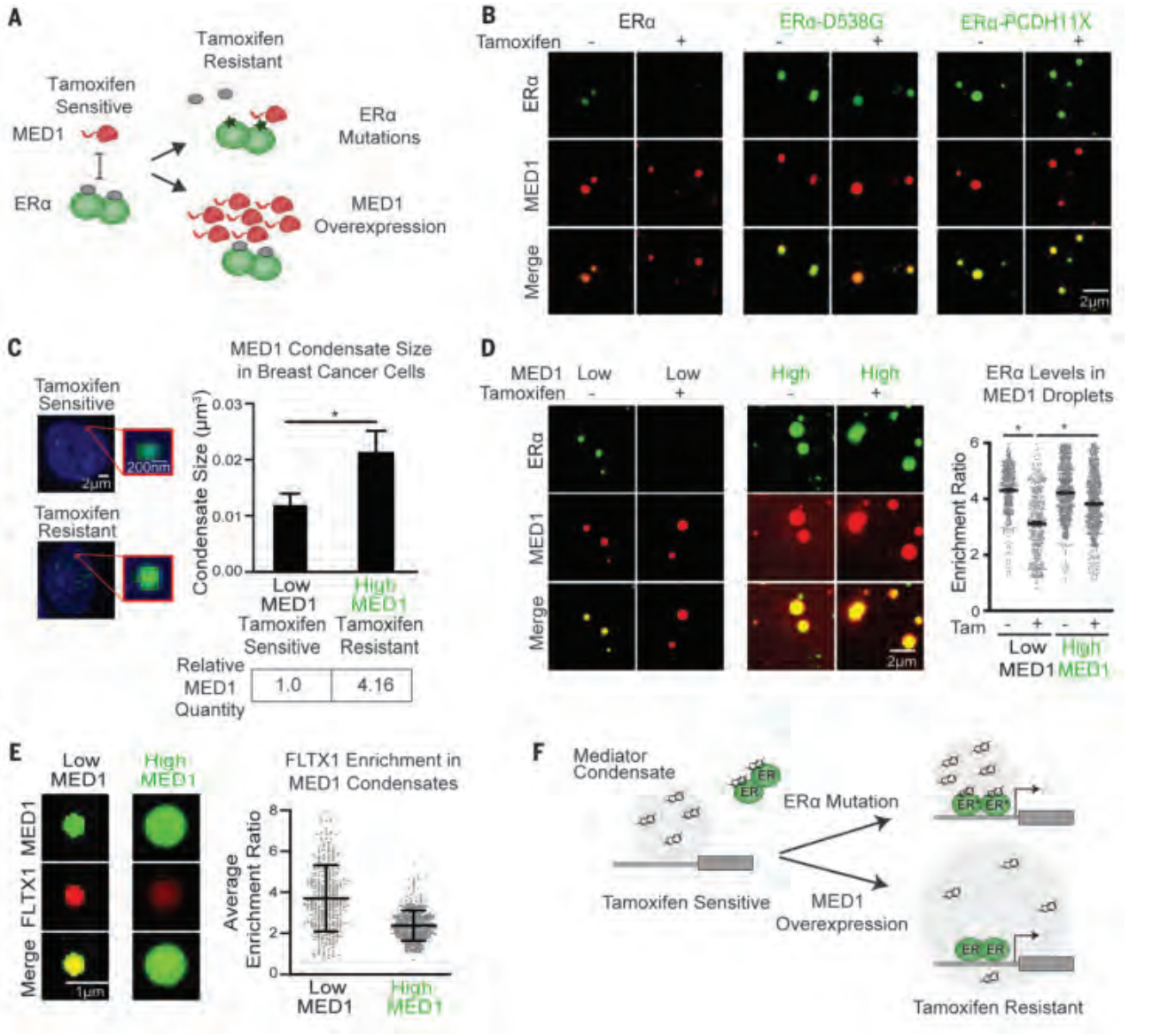

Fig. 4. Tamoxifen action and resistance in MED1 condensates. (**A**) Schematic showing tamoxifen resistance by ER mutation and MED1 overexpression in breast cancer. (**B**) In vitro droplet assay of the indicated form of GFP-tagged ER in the presence of estrogen with and without 100 μM tamoxifen. Droplets are formed in 125 mM NaCl and 10% PEG with a 10 μM concentration of each protein and 100 μM estrogen. (**C**) (Left) Immunofluorescence of MED1 in tamoxifen-sensitive (MCF7) and tamoxifen-resistant (TAMR7) ER$^+$ breast cancer cell lines imaged at 100× on a confocal fluorescent microscope. (Top right) Quantification of MED1 condensate size in breast cancer cells. (Bottom right) Relative quantities of MED1 in the indicated breast cancer cell line by Western blot. Error bars represent SEM. (**D**) In vitro droplet assays of ER in the presence of 100 μM estrogen with and without 100 μM tamoxifen with either 5 μM (low) or 20 μM (high) MED1. Droplets are formed with 5 μM ER in 125 mM NaCl and 10% PEG and imaged at 150× on a confocal fluorescent microscope. Error bars represent SEM. (**E**) In vitro droplet assay with either 5 μM (low) or 20 μM (high) MED1 with 100 μM FLTX1 in 125 mM NaCl and 10% PEG. Error bars represent SD. (**F**) Models for tamoxifen resistance caused by altered drug affinity (through ER mutation) or concentration (through MED1 overexpression) (see also figs. S22 to S30).

to express green fluorescent protein (GFP)–tagged marker proteins for each of the six nuclear condensates (figs. S17, A to F, and S18, A and B). When exposed to cisplatin, a selective and progressive reduction in MED1 condensates was observed (Fig. 3D and figs. S19, A and B, and S20A). Consistent with this, cisplatin treatment led to a preferential loss of MED1 chromatin immunoprecipitation sequencing (ChIP-seq) signal at SEs (Fig. 3E and fig. S21A). Furthermore, high-throughput sequencing data from platinated-DNA pulldown (*40*) revealed that cisplatin-modified DNA preferentially occurs at SEs, where MED1 is concentrated (*41*) (Fig. 3F). These results are consistent with reports that cisplatin preferentially modifies transcribed genes (*40*, *42*) and suggest that this effect is due to preferential condensate partitioning. Taken together, these results suggest a model in which cisplatin preferentially modifies SE DNA, which in turn leads to dissolution of these condensates. Previous studies have shown that diverse tumor cells become highly dependent on SE-driven oncogene expression (*43*–*47*), which might explain why platinum drugs, which are capable of general DNA modification, are effective therapeutics in diverse cancers (*48*).

We explored the behavior of another clinically important antineoplastic drug, tamoxifen, to assess whether its drug response and resistance were associated with partitioning in condensates (Fig. 4A). ERα incorporates into MED1 condensates in an estrogen-dependent manner in vitro (*12*); droplet assays confirmed this and revealed that the addition of tamoxifen leads to eviction of ERα from the MED1 condensates (Fig. 4B). We further investigated the effects of estrogen and tamoxifen on MED1 condensates in breast cancer cells, focusing on the *MYC* oncogene because of its prominent oncogenic role and responsiveness to estrogen (*49*). MED1 condensates were observed on the *MYC* oncogene in the ER^+ breast cancer cell line MCF7 (figs. S9A and S22, A to D). DNA fluorescence in situ hybridization with MED1 immunofluorescence revealed that estrogen enhances the formation of MED1 condensates at the *MYC* oncogene and that tamoxifen treatment reduces it (fig. S23, A and B). Artificial MED1 condensates without ER concentrated FLTX1 at the site of the condensate (fig. S24A), indicating that ER is not required for the partitioning of FLTX1 into MED1 condensates in cells. These results are consistent with the model that ERα interacts with MED1 condensates in an estrogen-dependent, tamoxifen-sensitive manner to drive oncogene expression in breast cancer cells.

The mechanisms that produce drug resistance can provide clues to drug activity in the clinical setting. Endocrine therapy and tamoxifen resistance are an enduring clinical challenge and are associated with multiple mechanisms, including ERα mutation and MED1 overexpression (Fig. 4A and fig. S25) (*50*–*54*). To investigate whether ERα mutations alter ERα behavior in condensates, we produced four patient-derived ERα mutant proteins and tested their partitioning in the presence of tamoxifen. In contrast to wild-type ERα, condensates composed of patient-derived ERα mutants and MED1 were not disrupted upon tamoxifen treatment (Fig. 4B and fig. S26, A and B). The ERα point mutations reduce the affinity for tamoxifen by ~10-fold (*51*), indicating that the drug concentration in the droplet is inadequate to evict these ER mutant proteins when this affinity is reduced.

MED1 overexpression is associated with tamoxifen resistance and poor prognosis in breast cancer (*50*), but it is not clear why overexpression of one subunit of the Mediator complex produces resistance. We considered the possibility that overexpressed MED1 is incorporated into transcriptional condensates, which contain clusters of Mediator molecules (*38*), thereby expanding their volumes and diluting the available tamoxifen (fig. S27A). We found that the tamoxifen-resistant breast cancer cell line TAMR7 (*55*), which was derived from the tamoxifen-sensitive cell line MCF7, produced fourfold elevated levels of the MED1 protein (fig. S27B). The volume of MED1-containing condensates was twofold larger in these cells (Fig. 4C and fig. S27C). When modeled in an in vitro droplet assay, we found that a fourfold increase in MED1 levels led to a commensurate increase in droplet size (fig. S28, A and B). Furthermore, we found that 100 μM tamoxifen prevented ERα incorporation into MED1 condensates (Fig. 4, B and D), but was much less effective in preventing ERα incorporation into the larger MED1 condensates produced with higher MED1 levels (Fig. 4D). To confirm that the levels of tamoxifen in the larger droplets are more dilute, we measured the enrichment of the fluorescent tamoxifen analog FLTX1 in MED1 droplets and found that the larger condensates had lower concentrations of the drug (Fig. 4E). These results were mirrored in cells, where a collection of tethered ERα molecules formed a MED1 condensate that was eliminated by tamoxifen, but when MED1 was overexpressed, tamoxifen was unable to dissociate the ERα-MED1 condensate (fig. S29A). Similarly, knockdown of MED1 in tamoxifen-resistant breast cancer cells sensitizes cells to tamoxifen (*50*, *54*). These results support a model of tamoxifen resistance where MED1 overexpression causes the formation of larger transcriptional condensates in which tamoxifen is diluted and is thereby less effective at dissociating ER from the condensate (Fig. 4F).

Our results show that drugs partition selectively into condensates, that this can occur through physicochemical properties that exist independently of their molecular targets, and that cells can develop resistance to drugs through condensate-altering mechanisms. This may explain the surprising observation that inhibition of global gene regulators such as BRD4 or CDK7 can have selective effects on oncogenes that have acquired large SEs (*45*); selective partitioning of inhibitors such as JQ1 and THZ1 into SE condensates will preferentially disrupt transcription at those loci. These results also have implications for the future development of efficacious disease therapeutics; effective target engagement will depend on measurable factors such as drug partitioning in condensates (fig. S30, A to D). Condensate assays of the type described here may thus help to optimize condensate partitioning, target engagement, and the therapeutic index of small-molecule drugs.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S30
References (*56*–*68*)
MDAR Reproducibility Checklist

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The *Zukunftskolleg* is a central research institution of the University of Konstanz for early career researchers in the natural sciences, humanities and social sciences. It is an integral component of the university's strategy „creative.together". The *Zukunftskolleg* promotes interdisciplinary discourse between distinguished researchers from Germany and abroad, and provides them with the resources to conduct their research independently and free from administrative constraints. The working language is English.

The *Zukunftskolleg* of the University of Konstanz offers:

three 5-year Research Fellowships (any discipline represented at the University of Konstanz)

(Salary Scale TV-L 14)

to develop and implement individual research projects and build up their own research group.

Fellowships will begin on May 1, 2021.

Reference number 2020/102

three 2-year Postdoctoral Fellowships (any discipline represented at the University of Konstanz)

(Salary Scale TV-L 13)

to develop and implement individual research projects. Conditionally on the submission of an external grant, the position can be extended for an additional year.

Fellowships will begin on April 1, 2021.

Reference number 2020/103

Zukunftskolleg

Applications, supporting materials and two letters of reference should be submitted in English **by 15 October 2020 11:00 am (Konstanz time) using the Online Application Platform** https://www.uni-konstanz.de/zukunftskolleg/online-application/.

Details concerning the application, required documents, and information about the Zukunftskolleg are available on our website: https://www.uni-konstanz.de/zukunftskolleg/.

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By **Akira Nishii**

Undergrads in charge

I entered the main conference room in our chemistry building, feeling nervous. I was the last to arrive, and I hastily took my place at a boat-shaped table alongside four other students, all undergrads like me. A group of professors sat across from us, chatting amicably among themselves. Scheduling this meeting—and getting more than one faculty member in a room at once—had taken me almost a month. Determined to make the most of their time, I collected my thoughts and skimmed the meeting agenda for a fifth time. Our group's goal, simple but unprecedented, was spelled out in bold at the top of my notes: "We want to build a university course from the bottom up."

We didn't know what would come out of the meeting. We wanted to create a course because we believed the introductory chemistry curriculum did not sufficiently prepare students for work in a research lab. But we worried the professors would see us as naïve children. What did we know about designing a course? Who were we to insist that the curriculum was inadequate?

I was in year two of my bachelor's degree, but my experience on campus went back further than that. I grew up near the university and had volunteered as a research assistant during my last 2 years of high school. A postdoc in a diabetes lab had taken me under his wing, teaching me how to extract DNA, perform Western blots, culture cells, and handle mice. I was grateful for the time he spent with me, sometimes doubting whether my presence amounted to more than a burden.

When I graduated, I decided to attend the university to study biology and chemical engineering. Fortunately, my earlier lab experience helped me secure another research assistant position, this time in a cancer lab. I went into the experience feeling more confident with my lab skills, which allowed me to focus on understanding the science.

At the same time, I observed that many of my peers were at a disadvantage because of their lack of research experience. Even after joining a lab, many of them had to spend months improving their lab techniques before they were able to contribute intellectually to research projects. That led me to brainstorm possible ways to help them.

I recruited other students who were interested in chemistry research, and we set out to convince faculty members that a new course was needed to address this issue. Our university's introductory chemistry course had a lab component, but it only covered a few lab techniques and wasn't tailored to students who wanted to get involved in chemistry research.

"Students should be advocates for their own education."

The new course that we had in mind would expose younger students to a diverse range of research skills, such as reading the literature and applying standard chemistry techniques. We didn't plan to ask faculty members to teach it; instead, we thought the course could be taught by older, more experienced undergraduate students.

When we met with the professors to discuss our proposed course, we assumed they would be skeptical. We were surprised to find they were instead open to our idea. One senior professor had to leave early, but before he did, he rearticulated our position and gave me a knowing wink. A younger professor then suggested that he could provide faculty oversight, and we started to discuss how we might secure funding and departmental resources.

Our course was added to the official course catalog for the following semester, and it was extremely popular, quickly exceeding the maximum enrollment capacity of 80 students. We designed the course ourselves, and two undergraduate students—both of whom were paid by the university—served as instructors. At the end of the semester, students and professors alike were happy with the outcome, so the course has continued to be offered in the years since.

It is all too common for undergraduate students to have little say in shaping their education. I'm grateful that the professors in our department were willing to listen and help us take the curriculum in a new direction, but I suspect this level of openness is rare. Students should be advocates for their own education—and universities should welcome this ambition with open arms. ■

Akira Nishii is an undergraduate student at the University of Michigan, Ann Arbor. Send your career story to SciCareerEditor@aaas.org.

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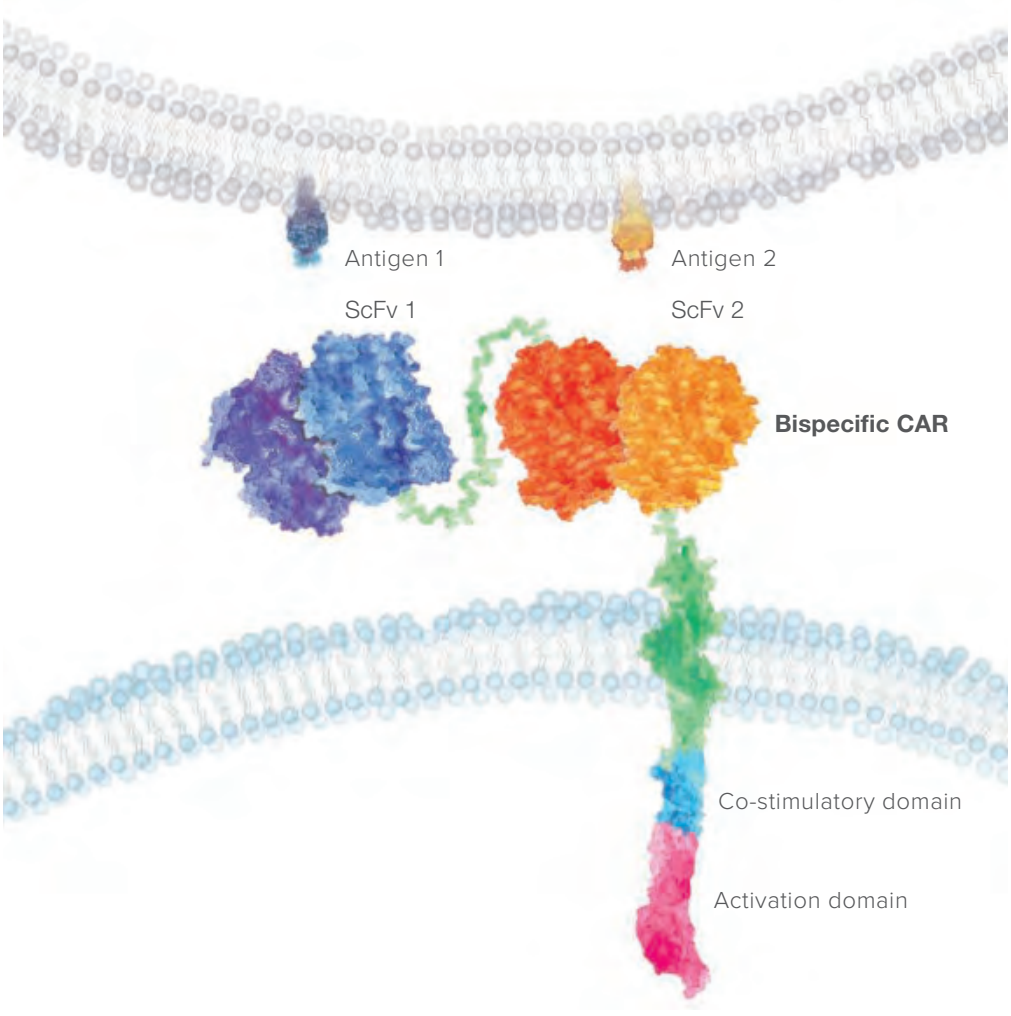

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